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=> d his ful

     FILE 'HCAPLUS' ENTERED AT 12:53:19 ON 09 FEB 2004
L1           81 SEA ABB=ON  ?GFAT?

     FILE 'REGISTRY' ENTERED AT 12:54:15 ON 09 FEB 2004
                E L-GLUTAMINE FRUCTOSE 6-PHOSPHATE AMINOTRANSFERASE/CN
                E L-GLUTAMINE-FRUCTOSE-6-PHOSPHATE AMINOTRANSFERASE/CN
                E NITRIC OXIDE SYNTHASE/CN
L2            1 SEA ABB=ON  "NITRIC OXIDE SYNTHASE"/CN
                E METHIONINE/CN
L3            2 SEA ABB=ON  METHIONINE/CN
                E AMINOPEPTIDASE/CN
L4            1 SEA ABB=ON  AMINOPEPTIDASE/CN
                E ASNSYN/CN
                E ASN SYN/CN
                E PFK/CN
L5            1 SEA ABB=ON  PFK/CN
                E P38/CN
                E P 38/CN
L6            1 SEA ABB=ON  "P 38"/CN
                E IKAPPA KINASE/CN
                E I-KAPPA KINASE/CN
                E KAPPA KINASE/CN
                E I KAPPA KINASE/CN
                E TBK1/CN
                E TBK 1/CN
                E MPKAP 2/CN
                E MPKAP1/CN
                E MPKAP2/CN
                E GTASE/CN
                E OGTASE/CN
                E CYCLOOXYGENASE/CN
L7            1 SEA ABB=ON  CYCLOOXYGENASE/CN
L8            7 SEA ABB=ON  L2 OR L3 OR L4 OR L5 OR L6 OR L7

     FILE 'HCAPLUS' ENTERED AT 12:59:29 ON 09 FEB 2004
L9          480 SEA ABB=ON  ?ENZYME?(W)?ACTIVITY?(W)?ASSAY?
L10         480 SEA ABB=ON  L9 AND (?ENZYME? OR ?ABZYME?)
L11           1 SEA ABB=ON  L10 AND ?LABEL?(W)?SUBSTRATE?
                D AU
L12           0 SEA ABB=ON  L10 AND ?DIFFERENT?(W)?CHARG?
L13           0 SEA ABB=ON  L10 AND ?SELECT?(W)?COUPL?
L14           1 SEA ABB=ON  L10 AND ION?(W)?EXCHANG?(W)?RESIN?
                D AU
L15         274 SEA ABB=ON  L10 AND (?DETERMINE? OR ?DETN? OR ?DETECT? OR
                ?MEASUR? OR ?CONTROL?)
L16          11 SEA ABB=ON  L15 AND (L8 OR ?GFAT? OR ?NITRIC?(W)?OXID?(W)?SYTHA
                S? OR ?METHIONINE? OR ?AMINOPEPTIDASE? OR ?ASNSYN? OR PFK? OR
                P38 OR I(W)?KAPPA?(W)?KINASE? OR ?TBK1? OR ?MPKAP2? OR ?GTASE?
                OR ?OGTASE? OR ?CYCLOOXYGENASE?)
L17           1 SEA ABB=ON  L16 AND (?STOP? OR ?COUPLE? OR ?BIND? OR ?BOUND?)
L18           3 SEA ABB=ON  L16 AND (?SOLUTION? OR ?FLUID? OR ?LIQUID? OR
                ?KINASE? OR ?MULTIPLE?(W)?WELL? OR ?AUTOMAT? OR ?MICROCHIP? OR
                ?IZOZYME? OR ?TIME?)
L19          11 SEA ABB=ON  L16 OR L17 OR L18
                D AU 1-11
L20          13 SEA ABB=ON  L19 OR L11 OR L14
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Gitomer 09/888,008

FILE 'MEDLINE, BIOSIS, EMBASE, JICST-EPLUS, JAPIO' ENTERED AT 13:09:14 ON 09 FEB 2004

FILE 'HCAPLUS' ENTERED AT 13:13:45 ON 09 FEB 2004

L21 3 SEA ABB=ON L15 AND ?NITRIC?(W)?OXID?(W)?SYNTHAS?

L22 0 SEA ABB=ON L21 AND (?STOP? OR ?COUPLE? OR ?BIND? OR ?BOUND?)

L23 1 SEA ABB=ON L21 AND (?SOLUTION? OR ?FLUID? OR ?LIZUID? OR ?KINASE? OR ?MULTIPLE?(W)?WELL? OR AUTOMAT? OR ?MICROCHIP? OR ?IZOZYME? OR ?TIME?)

L24 13 SEA ABB=ON L20 OR L21 OR L23 13 cits from CA Plus

FILE 'MEDLINE, BIOSIS, EMBASE, JICST-EPLUS, JAPIO' ENTERED AT 13:21:28 ON 09 FEB 2004

L25 67 SEA ABB=ON L20 OR L21 OR L23

L26 51 DUP REMOV L25 (16 DUPLICATES REMOVED) 51 cits from other d.b's

L27 0 SEA ABB=ON L26 AND (DIFFERENT?(W) CHARG? OR SELECT?(W) COUPL?)

Searched by Mary Jane Ruhl, x 22524 02/09/2004

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=> d que stat l24
L2              1 SEA FILE=REGISTRY ABB=ON  "NITRIC OXIDE SYNTHASE"/CN
L3              2 SEA FILE=REGISTRY ABB=ON  METHIONINE/CN
L4              1 SEA FILE=REGISTRY ABB=ON  AMINOPEPTIDASE/CN
L5              1 SEA FILE=REGISTRY ABB=ON  PFK/CN
L6              1 SEA FILE=REGISTRY ABB=ON  "P 38"/CN
L7              1 SEA FILE=REGISTRY ABB=ON  CYCLOOXYGENASE/CN
L8              7 SEA FILE=REGISTRY ABB=ON  L2 OR L3 OR L4 OR L5 OR L6 OR L7
L9            480 SEA FILE=HCAPLUS ABB=ON  ?ENZYME?(W)?ACTIVITY?(W)?ASSAY?
L10           480 SEA FILE=HCAPLUS ABB=ON  L9 AND (?ENZYME? OR ?ABZYME?)
L11             1 SEA FILE=HCAPLUS ABB=ON  L10 AND ?LABEL?(W)?SUBSTRATE?
L14             1 SEA FILE=HCAPLUS ABB=ON  L10 AND ION?(W)?EXCHANG?(W)?RESIN?
15            274 SEA FILE=HCAPLUS ABB=ON  L10 AND (?DETERMINE? OR ?DETN? OR
                  ?DETECT? OR ?MEASUR? OR ?CONTROL?)
L16            11 SEA FILE=HCAPLUS ABB=ON  L15 AND (L8 OR ?GFAT? OR ?NITRIC?(W)?O
                  XID?(W)?SYTHAS? OR ?METHIONINE? OR ?AMINOPEPTIDASE? OR
                  ?ASNSYN? OR PFK? OR P38 OR I(W)?KAPPA?(W)?KINASE? OR ?TBK1? OR
                  ?MPKAP2? OR ?GTASE? OR ?OGTASE? OR ?CYCLOOXYGENASE?)
L17             1 SEA FILE=HCAPLUS ABB=ON  L16 AND (?STOP? OR ?COUPLE? OR ?BIND?
                  OR ?BOUND?)
L18             3 SEA FILE=HCAPLUS ABB=ON  L16 AND (?SOLUTION? OR ?FLUID? OR
                  ?LIQUID? OR ?KINASE? OR ?MULTIPLE?(W)?WELL? OR ?AUTOMAT? OR
                  ?MICROCHIP? OR ?IZOZYME? OR ?TIME?)
L19            11 SEA FILE=HCAPLUS ABB=ON  L16 OR L17 OR L18
L20            13 SEA FILE=HCAPLUS ABB=ON  L19 OR L11 OR L14
L21             3 SEA FILE=HCAPLUS ABB=ON  L15 AND ?NITRIC?(W)?OXID?(W)?SYNTHAS?
L23             1 SEA FILE=HCAPLUS ABB=ON  L21 AND (?SOLUTION? OR ?FLUID? OR
                  ?LIZUID? OR ?KINASE? OR ?MULTIPLE?(W)?WELL? OR AUTOMAT? OR
                  ?MICROCHIP? OR ?IZOZYME? OR ?TIME?)
L24            13 SEA FILE=HCAPLUS ABB=ON  L20 OR L21 OR L23
```

=> d ibib abs 120 1-4

L24 ANSWER 1 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:184100 HCAPLUS
TITLE: Synthesis of GGPP analogs and their evaluation as **GGTase** I inhibitors
AUTHOR(S): Rickert, Emily Lynn
CORPORATE SOURCE: Department of Chemistry, Ohio Northern University, Ada, OH, 45810, USA
SOURCE: Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003 (2003), MEDI-133. American Chemical Society: Washington, D. C.
CODEN: 69DSA4
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Geranylgeranyl transferase I (**GGTase** I) prenylates Rho proteins, which are believed to be important in the metastasis of cancerous tumors. We have targeted several geranylgeranyl pyrophosphate (GGPP) analogs as potential inhibitors of **GGTase** I. Novel 7-substituted GGPP analogs were synthesized following a previously published method (Rawat and Gibbs, Organic Letters, 2002 in press). The eleven-step synthetic route produced 7-allyl geranylgeranyl pyrophosphate and 7-vinyl geranylgeranyl pyrophospate. These analogs will be evaluated using a fluorescence **enzyme activity assay** to **determine** inhibitory/substrate activity for **GGTase** I.

L24 ANSWER 2 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:180104 HCAPLUS
TITLE: Synthesis of GGPP analogs and their evaluation as **GGTase** I inhibitors
AUTHOR(S): Rickert, Emily Lynn
CORPORATE SOURCE: Department of Chemistry, Ohio Northern University, Ada, OH, 45810, USA
SOURCE: Abstracts of Papers, 225th ACS National Meeting, New Orleans, LA, United States, March 23-27, 2003 (2003), CHED-385. American Chemical Society: Washington, D. C.
CODEN: 69DSA4
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB Geranylgeranyl transferase I (**GGTase** I) prenylates Rho proteins, which are believed to be important in the metastasis of cancerous tumors. We have targeted several geranylgeranyl pyrophosphate (GGPP) analogs as potential inhibitors of **GGTase** I. Novel 7-substituted GGPP analogs were synthesized following a previously published method (Rawat and Gibbs, Organic Letters, 2002 in press). The eleven-step synthetic route produced 7-allyl geranylgeranyl pyrophosphate and 7-vinyl geranylgeranyl pyrophospate. These analogs will be evaluated using a fluorescence **enzyme activity assay** to **determine** inhibitory/substrate activity for **GGTase** I.

L24 ANSWER 3 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:450256 HCAPLUS
DOCUMENT NUMBER: 137:2733
TITLE: **Ion-exchange resin / enzyme activity assay**
INVENTOR(S): Karsten, Thomas P.; Currie, Mark G.; Moore, William M.
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 7 pp.

CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002072082	A1	20020613	US 2001-888008	20010622

PRIORITY APPLN. INFO.: US 2000-213354P P 20000622

AB The present invention relates to a rapid high-throughput **ion-exchange resin** assay for detg. **enzyme** activity. This novel assay uses a radiometric technique which separates the radioactive substrate from the product (or the radioactive product from the substrate) by exploiting the differences in the net charges of the mols. using **ion-exchange resin**. This assay is useful, for example, for studies of **enzyme** kinetics, the identification of functional sites in the **enzyme**, and in the automated screening of compd. libraries for pharmaceutical drug development.

L24 ANSWER 4 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:150567 HCAPLUS
DOCUMENT NUMBER: 136:337583
TITLE: The metIC operon involved in **methionine** biosynthesis in Bacillus subtilis is **controlled** by transcription antitermination
AUTHOR(S): Auger, Sandrine; Yuen, W. H.; Danchin, Antoine; Martin-Verstraete, Isabelle
CORPORATE SOURCE: Unite de Genetique des Genomes Bacteriens, Institut Pasteur, URA CNRS 2171, Paris, 75724, Fr.
SOURCE: Microbiology (Reading, United Kingdom) (2002), 148(2), 507-518
CODEN: MROBEO; ISSN: 1350-0872
PUBLISHER: Society for General Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB There are two major pathways for **methionine** biosynthesis in microorganisms. Little is known about these pathways in B. subtilis. The authors assigned a function to the metI (formerly yjcI) and metC (formerly yjcJ) genes of B. subtilis by complementing Escherichia coli metB and metC mutants, analyzing the phenotype of B. subtilis metI and metC mutants, and carrying out **enzyme activity assays**. These genes encode polypeptides belonging to the cystathionine .gamma.-synthase family of proteins. Interestingly, the MetI protein has both cystathionine .gamma.-synthase and O-acetylhomoserine thiolyase activities, whereas the MetC protein is a cystathionine .beta.-lyase. In B. subtilis, the transsulfuration and the thiolation pathways are functional in vivo. Due to its dual activity, the MetI protein participates in both pathways. The metI and metC genes form an operon, the expression of which is subject to S-dependent regulation. When the S source is SO42- or cysteine the transcription of this operon is high. Conversely, when the S source is **methionine** its transcription is low. An S-box sequence, which is located upstream of the metI gene, is involved in the regulation of the metIC operon. Northern blot expts. demonstrated the existence of 2 transcripts: a small transcript corresponding to the premature transcription termination at the terminator present in the S-box and a large one corresponding to transcription of the complete metIC operon. When **methionine** levels were limiting, the amt. of the full-length transcript increased. These results

substantiate a model of regulation by transcription antitermination.
REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L24 ANSWER 5 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:150165 HCAPLUS
DOCUMENT NUMBER: 137:166302
TITLE: Agrobacterium-mediated transformation of Vicia faba
AUTHOR(S): Bottinger, Petra; Steinmetz, Anke; Schieder, Otto; Pickardt, Thomas
CORPORATE SOURCE: Institute for Applied Genetics, Free University of Berlin, Berlin, 14195, Germany
SOURCE: Molecular Breeding (2001), 8(3), 243-254
CODEN: MOBRFL; ISSN: 1380-3743
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Among the major grain legume crops, Vicia faba belongs to those where the prodn. of transgenic plants has not yet convincingly been reported. We have produced stably transformed lines of faba bean with an Agrobacterium tumefaciens-mediated gene transfer system. Stem segments from aseptically germinated seedlings were inoculated with A. tumefaciens strains EHA101 or EHA105, carrying binary vectors conferring (1) uidA, (2) a mutant lysC gene, coding for a bacterial aspartate **kinase** insensitive to feedback **control** by threonine, and (3) the coding sequence for a **methionine**-rich sunflower 2S-albumin, each in combination with nptII as selectable marker. Kanamycin-resistant calluses were obtained on callus initiation medium at a frequency of 10-30%. Shoot regeneration was achieved on thidiazuron contg. medium in a second culture step. A subsequent transfer of shoots to BA-contg. medium was necessary for stem elongation and leaf development. Shoots were rooted or grafted onto young seedlings in vitro and mature plants were recovered. Mol. anal. confirmed the integration of the transgenes into the plant genome. Inheritance and expression of the foreign genes was demonstrated by Southern blot, PCR, western anal. and **enzyme activity assays**. Although at present the system is **time**-consuming and of relatively low efficiency, it represents a feasible approach for the prodn. of genetically engineered faba beans.
REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L24 ANSWER 6 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:720894 HCAPLUS
DOCUMENT NUMBER: 134:202495
TITLE: Effect of up-regulation of S-AdoMet synthetase on taxol-induced apoptosis in human breast cancer cells
AUTHOR(S): Chen, Lirong; Zheng, Shu; Fan, Weimin; Zhang, Suzhan
CORPORATE SOURCE: Cancer Institute, Zhejiang Medical University, Hangzhou, 310009, Peop. Rep. China
SOURCE: Chinese Journal of Cancer Research (1998), 10(4), 235-238
CODEN: CJCRFH; ISSN: 1000-9604
PUBLISHER: Chinese Journal of Cancer Research
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The gene regulation of taxol-induced apoptosis was investigated. Northern blot hybridization, **enzyme activity assay** of S-AdoMet synthetase and flow cytometry were performed in the investigation of expression in the mRNA level and biol. action of S-AdoMet synthetase in taxol-induced apoptosis in human breast cancer cell line (BCap37).

Up-regulation of S-AdoMet synthetase expression was resulted by taxol treatment and the expression peaked at 48 h. Moreover, the up-regulation of S-AdoMet synthetase was assocd. with cytotoxicity of anti-microbule agents including taxol and colchicine. Inhibition rate of S-AdoMet synthetase activity by 1% DMSO was 34% in taxol-treated cells and 14% in taxol-untreated cells compared to **control** groups, resp. Posttreatment with 1% DMSO following pretreatment with individual antitumor agent for 3 h promoted apoptotic cell death of taxol-, colchicine-, and adriamycin-treated Bcap37 cells. The induction of apoptosis enhanced by post-treatment with DMSO in taxol-treated cells is probably linked to its inhibition on **enzyme** activity of S-AdoMet synthetase, suggesting that the increased expression of S-AdoMet synthetase possibly plays an important role in protecting cells from DNA fragmentation in taxol-induced apoptosis.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L24 ANSWER 7 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1999:574736 HCAPLUS

DOCUMENT NUMBER: 131:252737

TITLE: Estrogen receptor-.alpha. gene transfer into bovine aortic endothelial cells induces eNOS gene expression and inhibits cell migration

AUTHOR(S): Tan, Enqing; Gurjar, Milind V.; Sharma, Ram V.; Bhalla, Ramesh C.

CORPORATE SOURCE: Department of Anatomy and Cell Biology, and The Cardiovascular Center, The University of Iowa College of Medicine, Iowa City, IA, 52242, USA

SOURCE: Cardiovascular Research (1999), 43(3), 788-797
CODEN: CVREAU; ISSN: 0008-6363

PUBLISHER: Elsevier Science B.V.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Objectives: It has been suggested that estrogen may improve endothelial cell function to delay the onset of atherosclerosis in pre-menopausal females, though its mechanism of action is not fully understood. The authors examd. the hypothesis that human estrogen receptor-.alpha. (ER.alpha.) gene transfection improves the endothelial cell function. Methods: A replication deficient adenoviral vector was used to transfect the ER.alpha. gene into bovine aortic endothelial cells (BAEC) and a GFP gene contg. vector was used as **control**. Expression of the eNOS gene was **detd.** by Northern blot anal. and **enzyme activity assay**; cell migration was assayed using a Transwell app.; and tyrosine phosphorylation of FAK was estd. by Western blot anal. Results: ER.alpha. gene transfection of endothelial cells produced a 2-3-fold increase in eNOS mRNA and protein levels as well as a significant increase ($P<0.05$) in NOS activity as **measured** by citrulline assay and nitrite accumulation in the media in response to bradykinin stimulation. Treatment of cells with estrogen blocking agent ICI 182780 inhibited eNOS induction in response to ER.alpha. transfection. ER.alpha. gene transfection significantly inhibited ($P<0.05$) bFGF-induced chemotactic migration of endothelial cells but increased cell attachment to fibronectin, laminin, and type I and IV collagens. ER.alpha. gene transfer also inhibited bFGF-stimulated tyrosine phosphorylation of FAK. Conclusion: the authors' results suggest that the atheroprotective effects of estrogen may in part be mediated by ER.alpha.-induced upregulation of eNOS gene expression and maintenance of endothelial cell function and integrity.

REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L24 ANSWER 8 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:392381 HCAPLUS
DOCUMENT NUMBER: 127:93584
TITLE: Overexpressed **nitric oxide synthase** in portal-hypertensive stomach of rat: a key to increased susceptibility to damage?
AUTHOR(S): Ohta, Masayuki; Tanoue, Kazuo; Tarnawski, Andrzej S.; Pai, Rama; Itani, Rabiha M.; Sander, Fred C.; Sugimachi, Keizo; Sarfeh, I. James
CORPORATE SOURCE: Department of Surgery, Department of Veterans Affairs Medical Center, Long Beach, CA, USA
SOURCE: Gastroenterology (1997), 112(6), 1920-1930
CODEN: GASTAB; ISSN: 0016-5085
PUBLISHER: Saunders
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Portal hypertension predisposes gastric mucosa to increased injury. The aim of this study was to **det.** whether overexpression of constitutive **nitric oxide synthase** (cNOS) is responsible for increased susceptibility of portal-hypertensive (PHT) gastric mucosa to damage. In gastric specimens from PHT and sham-operated rats, cNOS mRNA expression was **detd.** by Northern blotting and cNOS protein expression by Western blotting, immunohistochem., and **enzyme activity assay.** Extent of ethanol-induced gastric mucosal necrosis, mucosal blood flow, and gastric NOS activity in PHT and sham-operated rats was **detd.** after administration of N.omega.-nitro-L-arginine Me ester (L-NAME) or saline. CNOS mRNA level, cNOS **enzyme** activity, and fluorescence signals for cNOS were increased significantly in PHT rats compared with **controls.** Inhibition of overexpressed cNOS by L-NAME (5 mg/kg) significantly reduced ethanol-induced mucosal necrosis and normalized blood flow in PHT gastric mucosa, whereas this dose of L-NAME significantly increased mucosal necrosis in sham-operated rats. Portal hypertension activates the cNOS gene with overexpression of cNOS protein in endothelia of gastric mucosal vessels. Excessive NO prodn. by overexpressed cNOS may play an important role in the increased susceptibility of PHT gastric mucosa to damage.

L24 ANSWER 9 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1997:24103 HCAPLUS
DOCUMENT NUMBER: 126:58248
TITLE: Helicobacter pylori stimulates inducible **nitric oxide synthase** expression and activity in a murine macrophage cell line
AUTHOR(S): Wilson, Keith T.; Ramanujam, Kalathur S.; Mobley, Harry L. T.; Musselman, Robert F.; James, Stephen P.; Meltzer, Stephen J.
CORPORATE SOURCE: School Medicine, University Maryland, Baltimore, MD, USA
SOURCE: Gastroenterology (1996), 111(6), 1524-1533
CODEN: GASTAB; ISSN: 0016-5085
PUBLISHER: Saunders
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Helicobacter pylori uniquely colonizes the human stomach and produces gastric mucosal inflammation. High-output nitric oxide prodn. by inducible **nitric oxide synthase** (iNOS) is assocd. with immune activation and tissue injury. Because mononuclear

cells comprise a major part of the cellular inflammatory response to H. pylori infection, the ability of H. pylori to induce iNOS in macrophages was assessed. H. pylori prepns. were added to RAW 264.7 murine macrophages, and iNOS expression was assessed by Northern blot anal., **enzyme activity assay**, and NO2- release. Both whole H. pylori and French press lysates induced concn.-dependent NO2- prodn., with peak levels 20-fold above **control**. These findings were paralleled by marked increases in iNOS mRNA and **enzyme** activity levels. Inducible NOS expression was synergistically increased with interferon gamma, indicating that the H. pylori effect can be amplified by other macrophage-activating factors. Studies of lipopolysaccharide (LPS) content and polymyxin B inhibition of LPS suggested that the H. pylori effect was attributable to both LPS-dependent and -independent mechanisms. Thus, iNOS expression in macrophages is activated by highly stable H. pylori products and may play an important role in the pathogenesis of H. pylori-assocd. gastric mucosal disease.

REFERENCE COUNT: 52 THERE ARE 52 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L24 ANSWER 10 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER: 1995:22212 HCAPLUS
DOCUMENT NUMBER: 122:100179
TITLE: Purification of endothelin-1-inactivating peptidase from the rat kidney
AUTHOR(S): Janas, Jadwiga; Sitkiewicz, Dariusz; Pulawska, Maria F.; Warnawin, Krzysztof; Janas, Roman M.
CORPORATE SOURCE: Dep. Clin. Biochem., Natl. Inst. Cardiol., Warsaw, Pol.
SOURCE: Journal of Hypertension (1994), 12(4), 375-82
CODEN: JOHYD3; ISSN: 0263-6352
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Subcellular fractions of rat kidney, aorta, heart, lung, liver and blood cells were prepd. by differential centrifugation. Kidney membrane-**bound** peptidase was solubilized with Triton X-100, chromatographed on the diethylaminoethyl-cellulose, ultrafiltered through a membrane of relative mol. mass 100 000 cutoff and subjected to electrophoresis on a non-denaturing polyacrylamide gel. The **enzyme activity assay** was performed at pH 5.5 using [125I]-endothelin-1 as the substrate. The trichloroacetic acid pptn. test, an endothelin-1 immunoreactivity assay, reverse-phase high-performance **liq.** chromatog. and a receptor-**binding** assay were applied for the **detection** of degrdn. products. Results: High-activity endothelin-1-degrading peptidase coincided with the fraction from the kidney membranes of both Wistar-Kyoto and spontaneously hypertensive rats, but not with any other of the tissues that were studied. The membrane (0.5 .mu.g protein/assay) degraded [125I]-endothelin-1 (5-100 pmol/L) within a half-**time** of about 10 min at 37.degree.. The **enzyme** was purified to an apparent homogeneity with non-denaturing gel electrophoresis, by which it was identified as a low-mobility (Rf 0.07) protein fraction of high relative mol. mass (>250 000). The optimum pH was 5.5, with a little activity found outside the range 5.0-7.0. The activity of the peptidase was inhibited by 0.5 mmol/L 1,10-phenanthroline (half-maximal inhibitory concn. 0.03 mmol/L), and by 1 mmol/L EDTA, implicating a **metalloenzyme**. Bestatin, puromycin, phenylmethylsulfonyl fluoride and thiorphan were without effect. Unlabeled endothelin-1 inhibited the degrdn. of [125I]-endothelin-1 (half-maximal inhibitory concn. 100 nmol/L), whereas 100 .mu.mol/L **methionine** enkephalin or angiotensin I did not. High-performance **liq.** chromatog. analyses of the [125I]-endothelin-1 incubated with

purified peptidase revealed a **time**-dependent accumulation of one major radioactive fraction that was sol. in trichloroacetic acid. This product (or products) was not further hydrolyzed. It did not react with the endothelin antibodies or with the specific, myocardial membrane receptors. The data suggest that the rat kidney contains an acidic metalloproteinase of high relative mol. mass that is able to hydrolyze endothelin-1 rapidly and efficiently in vitro. The **enzyme** may participate in the inactivation of circulating or tissue endothelins, or both.

L24 ANSWER 11 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1992:220884 HCAPLUS
DOCUMENT NUMBER: 116:220884
TITLE: **Enzyme** activities in waste water and activated sludge
AUTHOR(S): Nybroe, Ole; Joergensen, Per Elberg; Henze, Mogens
CORPORATE SOURCE: Water Qual. Inst., Hoersholm, DK-2970, Den.
SOURCE: Water Research (1992), 26(5), 579-84
CODEN: WATRAG; ISSN: 0043-1354
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The potential of selected **enzyme activity assays** to **det.** microbial abundance and heterotrophic activity in wastewater and activated sludge was evaluated. In wastewater, esterase and dehydrogenase activities were correlated with microbial abundance **measured** as colony forming units of heterotrophic bacteria. A panel of 4 **enzyme activity assays**, .alpha.-glucosidase, alanine-**aminopeptidase**, esterase, and dehydrogenase were used to characterize activated sludge and anaerobic hydrolysis sludge from a pilot scale plant. The enzymic activity profiles were distinctly different, suggesting that microbial populations were different, or had different physiol. properties, in the 2 types of sludge. **Enzyme** activity profiles in activated sludge from 4 full-scale plants seemed to be highly influenced by the compn. of the inlet. Addn. of hydrolyzed starch was, for instance, reflected in a high .alpha.-glucosidase activity. However, no obvious correlations between specific process parameters and **enzyme** activities were found.

L24 ANSWER 12 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:587674 HCAPLUS
DOCUMENT NUMBER: 109:187674
TITLE: A role for glyceraldehyde-3-phosphate dehydrogenase in the development of thermotolerance in Xenopus laevis embryos
AUTHOR(S): Nickells, Robert W.; Browder, Leon W.
CORPORATE SOURCE: Dep. Biol. Sci., Univ. Calgary, Calgary, AB, T2N 1N4, Can.
SOURCE: Journal of Cell Biology (1988), 107(5), 1901-9
CODEN: JCLBA3; ISSN: 0021-9525
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The glycolytic **enzyme** glyceraldehyde-3-phosphate dehydrogenase ([EC 1.2.1.12] GAPDH) showed a significant increase after heat shock, which correlated with an accumulation of GAPDH in heat-shocked Xenopus embryos as **detected** by immunoblotting. Increases in GAPDH-specific activity were variable, however, and were inversely proportional to the levels of specific activity in **control** embryos; i.e., constitutive **enzyme** activity. **Control** embryos exhibited a single **isoenzyme** of GAPDH, whereas heat-shocked embryos exhibited 2 **isoenzymes** of GAPDH. When

these **isoenzymes** are labeled with [35S]**methionine**, sepd. by nondenaturing gel electrophoresis, and analyzed by fluorog., a heat-shock protein was found to comigrate with the **isoenzyme** unique to the heat-shocked sample. **Enzyme activity assays** at different temps. suggest that this **isoenzyme** has optimum enzymic activity only at heat-shock temp. A 35-kD heat-shock protein (hsp35) was correlated with GAPDH by using the following evidence: this hsp comigrates with GAPDH on one-dimensional SDS polyacrylamide gels; heat-enhanced increases in GAPDH specific activity correlate with hsp35 synthesis; and hsp35 and GAPDH had similar peptide mpas. This relationship also provides a compelling explanation for the restriction of hsp35 synthesis to the vegetal hemisphere cells of heat-shocked early gastrulae reported previously.

L24 ANSWER 13 OF 13 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1972:548884 HCAPLUS
DOCUMENT NUMBER: 77:148884
TITLE: Radiochemical method for cellulose-degrading **enzymes activity assay**
AUTHOR(S): Kolev, D.; Popov, P.; Gros, G.
CORPORATE SOURCE: Cent. Res. Lab. Radiobiol. Control Harmful Rodents, Acad. Agric. Sci., Sofia, Bulg.
SOURCE: Pharmazie (1972), 27(8), 545-6
CODEN: PHARAT; ISSN: 0031-7144
DOCUMENT TYPE: Journal
LANGUAGE: English

AB An improved cellulose-degrading **enzymes** activity (CDEA) assay was developed which increased precision and reduced assay time. Hydrolysis of 14C-labeled cellulose substrate of known sp. activity catalyzed by a cellulase (Merck) of known activity (90 munits/mg) was followed kinetically. Results were given as dpm. (decay/min.). Enzymic reactions followed zero-order kinetics during the first 20 min. of incubation. The initial velocity (V0) of the enzymic reaction was directly proportional to the **enzyme** concn. In the detn. of the CDEA values, a relative radiation unit (RRU) was introduced, representing the amt. of **enzyme** which releases 1 nCi (2.22 .times. 103 dpm) H2O-sol. radioactivity per 1 mg 14C-**labeled substrate** with a sp. activity of 1 .mu.Ci/mg after incubating at 37.degree. for 1 min.

```
=> d que stat l26
L2              1 SEA FILE=REGISTRY ABB=ON  "NITRIC OXIDE SYNTHASE"/CN
L3              2 SEA FILE=REGISTRY ABB=ON  METHIONINE/CN
L4              1 SEA FILE=REGISTRY ABB=ON  AMINOPEPTIDASE/CN
L5              1 SEA FILE=REGISTRY ABB=ON  PFK/CN
L6              1 SEA FILE=REGISTRY ABB=ON  "P 38"/CN
L7              1 SEA FILE=REGISTRY ABB=ON  CYCLOOXYGENASE/CN
L8              7 SEA FILE=REGISTRY ABB=ON  L2 OR L3 OR L4 OR L5 OR L6 OR L7
L9            480 SEA FILE=HCAPLUS ABB=ON  ?ENZYME?(W)?ACTIVITY?(W)?ASSAY?
L10           480 SEA FILE=HCAPLUS ABB=ON  L9 AND (?ENZYME? OR ?ABZYME?)
L11             1 SEA FILE=HCAPLUS ABB=ON  L10 AND ?LABEL?(W)?SUBSTRATE?
L14             1 SEA FILE=HCAPLUS ABB=ON  L10 AND ION?(W)?EXCHANG?(W)?RESIN?
L15           274 SEA FILE=HCAPLUS ABB=ON  L10 AND (?DETERMINE? OR ?DETN? OR
                  ?DETECT? OR ?MEASUR? OR ?CONTROL?)
L16            11 SEA FILE=HCAPLUS ABB=ON  L15 AND (L8 OR ?GFAT? OR ?NITRIC?(W)?O
                  XID?(W)?SYTHAS? OR ?METHIONINE? OR ?AMINOPEPTIDASE? OR
                  ?ASNSYN? OR PFK? OR P38 OR I(W)?KAPPA?(W)?KINASE? OR ?TBK1? OR
                  ?MPKAP2? OR ?GTASE? OR ?OGTASE? OR ?CYCLOOXYGENASE?)
L17             1 SEA FILE=HCAPLUS ABB=ON  L16 AND (?STOP? OR ?COUPLE? OR ?BIND?
                  OR ?BOUND?)
L18             3 SEA FILE=HCAPLUS ABB=ON  L16 AND (?SOLUTION? OR ?FLUID? OR
                  ?LIQUID? OR ?KINASE? OR ?MULTIPLE?(W)?WELL? OR ?AUTOMAT? OR
                  ?MICROCHIP? OR ?IZOZYME? OR ?TIME?)
L19            11 SEA FILE=HCAPLUS ABB=ON  L16 OR L17 OR L18
L20            13 SEA FILE=HCAPLUS ABB=ON  L19 OR L11 OR L14
L21             3 SEA FILE=HCAPLUS ABB=ON  L15 AND ?NITRIC?(W)?OXID?(W)?SYNTHASE
                  ?LIZUID? OR ?KINASE? OR ?MULTIPLE?(W)?WELL? OR AUTOMAT? OR
                  ?MICROCHIP? OR ?IZOZYME? OR ?TIME?)
L25            67 SEA L20 OR L21 OR L23
L26            51 DUP REMOV L25 (16 DUPLICATES REMOVED)
```

=> d ibib abs l26 1-51

L26 ANSWER 1 OF 51 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2003345544 EMBASE
TITLE: Enhancement of **aminopeptidase** A expression during Angiotensin II-induced choriocarcinoma cell proliferation through AT(1) receptor involving protein **kinase** C- and mitogen-activated protein **kinase**-dependent signaling pathway.
AUTHOR: Ino K.; Uehara C.; Kikkawa F.; Kajiyama H.; Shibata K.; Suzuki T.; Khin E.E.; Ito M.; Takeuchi M.; Itakura A.; Mizutani S.
CORPORATE SOURCE: Dr. K. Ino, Dept. of Obstetrics and Gynecology, Nagoya Univ. Grad. Sch. of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan. kazuino@med.nagoya-u.ac.jp
SOURCE: Journal of Clinical Endocrinology and Metabolism, (1 Aug 2003) 88/8 (3973-3982).
Refs: 46
ISSN: 0021-972X CODEN: JCEMAZ
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 003 Endocrinology
021 Developmental Biology and Teratology
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Angiotensin II (Ang II) is a bioactive peptide of the renin-angiotensin system, exerting its actions not only as a vasoconstrictor, but also as a growth promoter. In human placenta, type 1 Ang II receptors (AT(1)R) are predominantly expressed in trophoblasts, and we previously reported that **aminopeptidase** A (APA), a cell surface peptidase that converts Ang II to Ang III, is also expressed in both normal and neoplastic trophoblasts. However, the roles of Ang II and APA in trophoblast function remain to be clarified. In the present study we examined the effects of Ang II on proliferation and APA expression in trophoblast-like BeWo choriocarcinoma cells. Treatment of BeWo cells with Ang II significantly increased DNA synthesis in a dose-dependent manner. Ang II also enhanced APA mRNA and cell surface expression in BeWo cells analyzed by Northern blotting, flow cytometry, and **enzyme activity assay**. The Ang II-induced proliferation and APA up-regulation were blocked by the AT (1)R antagonist candesartan, but not by the AT(2)R antagonist PD123319. Furthermore, these Ang II effects were abolished by the protein **kinase** C inhibitor bisindolylmaleimide I and the MAPK inhibitor PD98059. Immunohistochemistry using choriocarcinoma tissues demonstrated that APA was expressed on the cell surface of AT(1)R-positive cytotrophoblastic cells in vivo. With these findings we demonstrate that Ang II stimulates the proliferation of trophoblastic cells via AT(1)R that are linked to protein **kinase** C/MAPK-dependent signaling pathways, and that the Ang II-degrading **enzyme** APA is up-regulated during Ang II-induced cell proliferation. These observations suggest the possible regulatory mechanism by the local renin-angiotensin system, especially the Ang II-AT(1)R-APA system, for the growth of human choriocarcinoma cells.

L26 ANSWER 2 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:128507 BIOSIS
DOCUMENT NUMBER: PREV200300128507
TITLE: **Cyclooxygenase** 2 inhibits SAPK activation in neuronal apoptosis.

AUTHOR(S): Miller, Bradley; Chang, Yu-Wen E.; Sorokin, Andrey [Reprint Author]
CORPORATE SOURCE: Department of Medicine, Cardiovascular Research Center, Medical College of Wisconsin, Milwaukee, WI, 53226-0509, USA
sorokin@mcw.edu
SOURCE: Biochemical and Biophysical Research Communications, (January 24 2003) Vol. 300, No. 4, pp. 884-888. print.
CODEN: BBRCA9. ISSN: 0006-291X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Mar 2003
Last Updated on STN: 5 Mar 2003

AB **Cyclooxygenase** 2 (COX-2) expressed in cultured neuronal PC12 cells under inducible promoter protects cells from trophic withdrawal apoptosis. Stimulation of SAPK is thought to play a significant role in initiation of PC12 cell death. We have therefore examined whether COX-2 expression inhibits trophic withdrawal-mediated activation of SAPK. SAPK activity increased during the first 6 h after NGF removal in mock-transfected PC12 cells. COX-2 expression attenuated the increase of SAPK, as **detected** by Western blot analysis with phosphorylation state specific anti-SAPK antibodies and by SAPK activity assays. We propose that COX-2 attenuated SAPK activation by preventing activation of nNOS, which occurs, as we have shown before, via COX-2-mediated expression of dynein light chain (DLC). Activation of SAPK in neuronal cell death was attenuated by DLC expression. These observations support a role for NO production and SAPK activation in the neuronal death mechanisms.

L26 ANSWER 3 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:488248 BIOSIS
DOCUMENT NUMBER: PREV200300490145
TITLE: Expression of **nitric oxide synthase** in the preoptic-hypothalamo-hypophyseal system of the teleost Oreochromis niloticus.
AUTHOR(S): Bordieri, Loredana; Persichini, Tiziana; Venturini, Giorgio; Cioni, Carla [Reprint Author]
CORPORATE SOURCE: Department of Animal and Human Biology, 'La Sapienza' University, Via A. Borelli 50, IT-00161, Rome, Italy
carla.cioni@uniroma1.it
SOURCE: Brain Behavior and Evolution, (August 2003) Vol. 62, No. 1, pp. 43-55. print.
CODEN: BRBEBE. ISSN: 0006-8977.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Oct 2003
Last Updated on STN: 22 Oct 2003

AB In the present study, we have analyzed the expression of **nitric oxide synthase** (NOS) in the preoptic-hypothalamo-hypophyseal system of the teleost Oreochromis niloticus. The assay for **enzyme** activity demonstrated that a constitutive NOS activity is present both in soluble and particulate fractions of the homogenates of diencephalons. Western blot analysis using an antibody against the N-terminus of human nNOS revealed two bands both in the supernatant and in the pellet. One band co-migrates at approximately 150 kDa with that **detected** in the rat cerebellum homogenates and presumably corresponds to neuronal NOS (nNOS) of mammals. The additional band, which migrates at approximately 180 kDa, might be attributed to an alternatively spliced nNOS isoform. Using NADPH diaphorase (NADPHd) histochemistry in combination with NOS immunohistochemistry, nNOS expression has been **detected** in preoptic nuclei, hypophysiotrophic nuclei of the

ventral hypothalamus, and the pituitary gland. Various degrees of dissociation of NADPHd activity and nNOS immunoreactivity have been **detected** that could be attributed to the expression of different subtypes of nNOS in the preoptic/hypothalamo/hypophysial system of tilapia. In this paper, we also investigated the colocalization of nNOS with arginine-vasotocin (AVT) by means of immunolabeling of consecutive sections. Results suggest that NO may be colocalized with AVT in a subpopulation of neurosecretory neurons. Present findings suggest that nitric oxide (NO) is implicated in the modulation of hormone release in teleosts in a similar way to mammals.

L26 ANSWER 4 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:477519 BIOSIS
DOCUMENT NUMBER: PREV200200477519
TITLE: Isoform-selective interaction of **cyclooxygenase**-2 with indomethacin amides studied by real-**time** fluorescence, inhibition kinetics, and site-directed mutagenesis.
AUTHOR(S): Timofeevski, Sergei L.; Prusakiewicz, Jeffery J.; Rouzer, Carol A.; Marnett, Lawrence J. [Reprint author]
CORPORATE SOURCE: Departments of Biochemistry and Chemistry, Center in Molecular Toxicology, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN, 37232, USA
marnett@toxicology.mc.vanderbilt.edu
SOURCE: Biochemistry, (July 30, 2002) Vol. 41, No. 30, pp. 9654-9662. print.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 11 Sep 2002
Last Updated on STN: 11 Sep 2002

AB Conversion of carboxylate-containing nonsteroidal antiinflammatory drugs, such as indomethacin, to esters or amides provides potent and selective inhibitors of **cyclooxygenase**-2 (COX-2) (Kalgutkar et al. (2000) Proc. Natl. Acad. Sci. U.S.A. 97, 925-930). Synthesis of cinnamyl- or coumarinyl-substituted ethanolamide derivatives of indomethacin produced fluorescent probes of inhibitor interaction with COX-2 and COX-1. **Binding** of either derivative to apoCOX-2 or apoCOX-1 resulted in a rapid, reversible enhancement of fluorescence. Following this rapid phase, a slow additional increase in fluorescence was observed with apoCOX-2 but not with apoCOX-1. The slow, COX-2-specific increase in fluorescence was prevented or reversed by addition of the nonfluorescentCOX inhibitor (S)-flurbiprofen. Detailed kinetic studies of the interaction of the coumarinyl derivative with COX-2 showed that the observed changes in fluorescence could be described by two sequential equilibria, the first of which is rapid, reversible, and bimolecular in the forward direction. The second equilibrium is slower, reversible, and unimolecular in both directions. The forward rate constant for the slow equilibrium **determined** by fluorescence enhancement ((3.1 +- 0.6) X 10-3 s-1) corresponded closely to the forward rate constant for inhibition of COX-2 activity ((6.8 +- 2.3) X 10-3 s-1), suggesting that the slow fluorescence enhancement is associated with selective COX-2 inhibition. Site-directed mutagenesis indicated that residues in the carboxylate-**binding** region of the COX-2 active site (Arg-120, Tyr-355, and Glu-524) are critical for the **binding** of the indomethacin conjugates that leads to slow fluorescence enhancement and **cyclooxygenase** inhibition. The indomethacin conjugates described herein represent powerful tools for the investigation of a novel class of selective inhibitors of COX-2.

L26 ANSWER 5 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 1
ACCESSION NUMBER: 2002:542105 BIOSIS
DOCUMENT NUMBER: PREV200200542105
TITLE: Oxidative metabolism modulates signal transduction and micronucleus formation in bystander cells from alpha-particle-irradiated normal human fibroblast cultures.
AUTHOR(S): Azzam, Edouard I.; de Toledo, Sonia M.; Spitz, Douglas R.; Little, John B. [Reprint author]
CORPORATE SOURCE: Department of Cancer Cell Biology, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA, 02115, USA
SOURCE: Cancer Research, (October 1, 2002) Vol. 62, No. 19, pp. 5436-5442. print.
CODEN: CNREA8. ISSN: 0008-5472.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 23 Oct 2002
Last Updated on STN: 23 Oct 2002

AB The role of oxidative metabolism in the up-regulation/activation of stress-inducible signaling pathways as well as induction of micronucleus formation in bystander cells was investigated. By immunoblotting and in situ immunofluorescence, active Cu-Zn superoxide dismutase (SOD) **enzyme** and active catalase **enzyme** were shown to inhibit the up-regulation of p21Waf1 as well as the induction of micronucleus formation in bystander cells from confluent cultures of normal human diploid fibroblasts irradiated with 0.3-3 cGy of alpha-particles. **Enzyme activity assays** indicated that exogenous SOD became significantly associated with the cells. Reactive oxygen species apparently derived from a flavin-containing oxidase **enzyme** (presumably an NAD(P)H-oxidase) appeared to be major contributors to the bystander-induced up-regulation of p53 and p21Waf1 as well as micronucleus formation, as evidenced by the inhibition of these effects with diphenyliodonium. Rapid activation of nuclear factor kappaB, Raf-1, extracellular signal-regulated **kinase** 1/2, c-Jun NH2-terminal **kinase**, and **p38** mitogen-activated protein **kinase** and their downstream effectors activator protein 1, ELK-1, p90RSK, and activating transcription factor 2 was also observed in cultures exposed to very low fluences of alpha-particles. Significant attenuation in the activation of these **kinases** and transcription factors occurred in irradiated cultures treated with either SOD or catalase. Overall, these results support the hypothesis that superoxide and hydrogen peroxide produced by flavin-containing oxidase **enzymes** mediate the activation of several stress-inducible signaling pathways as well as micronucleus formation in bystander cells from cultures of human cells exposed to low fluences of alpha-particles.

L26 ANSWER 6 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:101564 BIOSIS
DOCUMENT NUMBER: PREV200300101564
TITLE: Marker for real-**time** analysis of caspase activity in intact cells.
AUTHOR(S): Lee, Pui; Beem, Elaine; Segal, Mark S. [Reprint Author]
CORPORATE SOURCE: P.O. Box 100224, Gainesville, FL, 32610, USA
segalms@medicine.ufl.edu
SOURCE: BioTechniques, (December 2002) Vol. 33, No. 6, pp. 1284-1291. print.
ISSN: 0736-6205 (ISSN print).
DOCUMENT TYPE: Article
LANGUAGE: English

ENTRY DATE: Entered STN: 19 Feb 2003
Last Updated on STN: 19 Feb 2003

AB Apoptosis, or programmed cell death, is an important regulator of growth, development, defense, and homeostasis in multicellular organisms. A family of cysteine proteases known as caspases is central to many apoptotic pathways, and thus **detection** of their activity offers an effective means to assess apoptosis. However, currently available methods only allow the evaluation of in vivo caspase activity at a given **time** point or over a few hours. To assess the activity over extended periods of **time**, we designed a novel, real-**time**, in vivo marker that utilizes the N-end rule degradation pathway to allow the **detection** of caspase activity as reflected by increasing enhanced GFP (EGFP) stability. The marker has an N-terminal arginine in the absence of caspase activity and is rapidly degraded. In vivo caspase activity removes the marker's N-terminal arginine residue, leaving an EGFP with an N-terminal **methionine** that results in stable fluorescence. In our study, the marker accurately depicted an increase in caspase activity in apoptotic cells and also **detected** significant endogenous caspase activity in non-apoptotic cells. The downstream effects of this endogenous activity **detected** in intact, non-apoptotic cells must be regulated by the cell preventing apoptosis. These studies also demonstrate the feasibility of using the N-end rule to study endogenous enzymatic activities other than those associated with proteasomal degradation.

L26 ANSWER 7 OF 51 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002147697 MEDLINE
DOCUMENT NUMBER: 21822507 PubMed ID: 11832514
TITLE: The metIC operon involved in **methionine** biosynthesis in Bacillus subtilis is **controlled** by transcription antitermination.
AUTHOR: Auger Sandrine; Yuen W H; Danchin Antoine; Martin-Verstraete Isabelle
CORPORATE SOURCE: Unite de Genetique des Genomes Bacteriens, Institut Pasteur, URA CNRS 2171, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France.
SOURCE: MICROBIOLOGY, (2002 Feb) 148 (Pt 2) 507-18. Journal code: 9430468. ISSN: 1350-0872.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200204
ENTRY DATE: Entered STN: 20020308
Last Updated on STN: 20020426
Entered Medline: 20020425

AB There are two major pathways for **methionine** biosynthesis in micro-organisms. Little is known about these pathways in Bacillus subtilis. The authors assigned a function to the metI (formerly yjcI) and metC (formerly yjcJ) genes of B. subtilis by complementing Escherichia coli metB and metC mutants, analysing the phenotype of B. subtilis metI and metC mutants, and carrying out **enzyme activity assays**. These genes encode polypeptides belonging to the cystathionine gamma-synthase family of proteins. Interestingly, the MetI protein has both cystathionine gamma-synthase and O-acetylhomoserine thiolyase activities, whereas the MetC protein is a cystathionine beta-lyase. In B. subtilis, the transsulfuration and the thiolation pathways are functional in vivo. Due to its dual activity, the MetI protein participates in both pathways. The metI and metC genes form an operon, the expression of which is subject to sulfur-dependent regulation.

When the sulfur source is sulfate or cysteine the transcription of this operon is high. Conversely, when the sulfur source is **methionine** its transcription is low. An S-box sequence, which is located upstream of the metI gene, is involved in the regulation of the metIC operon. Northern blot experiments demonstrated the existence of two transcripts: a small transcript corresponding to the premature transcription termination at the terminator present in the S-box and a large one corresponding to transcription of the complete metIC operon. When **methionine** levels were limiting, the amount of the full-length transcript increased. These results substantiate a model of regulation by transcription antitermination.

L26 ANSWER 8 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:239061 BIOSIS
DOCUMENT NUMBER: PREV200200239061
TITLE: Tandem mass spectrometric assay for the determination of carnitine palmitoyltransferase II activity in muscle tissue.
AUTHOR(S): Rettinger, Armin; Gempel, Klaus; Hofmann, Sabine; Gerbitz, Klaus-Dieter; Bauer, Matthias F. [Reprint author]
CORPORATE SOURCE: Molekulare Diagnostik und Mitochondriale Genetik, Institut fuer Klinische Chemie, KH Muenchen-Schwabing, Koelner Platz 1, D-80804, Munich, Germany
bauer@bio.med.uni-muenchen.de
SOURCE: Analytical Biochemistry, (March 15, 2002) Vol. 302, No. 2, pp. 246-251. print.
CODEN: ANBCA2. ISSN: 0003-2697.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Apr 2002
Last Updated on STN: 10 Apr 2002

AB Carnitine palmitoyltransferase II (CPT-II) mediates the import of long-chain fatty acids into the mitochondrial matrix for subsequent beta-oxidation. Defects of CPT-II manifest as a severe neonatal hepatocardiomuscular form or as a mild muscular phenotype in early infancy or adolescence. CPT-II deficiency is diagnosed by the determination of **enzyme** activity in tissues involving the time-dependent conversion of radiolabeled CPT-II substrates (isotope-exchange assays) or the formation of chromogenic reaction products. We have established a mass spectrometric assay (MS/MS) for the determination of CPT-II activity based on the stoichiometric formation of acetylcarnitine in a coupled reaction system. In this single-tube reaction system palmitoylcarnitine is converted by CPT-II to free carnitine, which is subsequently esterified to acetylcarnitine by carnitine acetyltransferase. The formation of acetylcarnitine directly correlates with the CPT-II activity. Comparison of the MS/MS method (y) with our routine spectrophotometric assay (x) revealed a linear regression of $y=0.58x+0.12$ ($r=0.8369$). Both assays allow one to unambiguously detect patients with the muscular form of CPT-II deficiency. However, the higher specificity and sensitivity as well as the avoidance of the drawbacks inherent in the use of **radiolabeled substrates** make this mass spectrometric method most suitable for the determination of CPT-II activity.

L26 ANSWER 9 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:459117 BIOSIS
DOCUMENT NUMBER: PREV200200459117
TITLE: Application of isotopic ratio mass spectrometry for the in vitro determination of demethylation activity in human liver microsomes using N-methyl-13C-**labeled substrates**.

AUTHOR(S): Grand, Florence [Reprint author]; Kilinc, Izzet [Reprint author]; Sarkis, Albert [Reprint author]; Guitton, Jerome [Reprint author]
CORPORATE SOURCE: Federation de Biochimie, Laboratoire de Biochimie C, Hopital E. Herriot, 69437, Lyon Cedex 03, France
SOURCE: Analytical Biochemistry, (July 15, 2002) Vol. 306, No. 2, pp. 181-187. print.
CODEN: ANBCA2. ISSN: 0003-2697.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Aug 2002
Last Updated on STN: 28 Aug 2002

AB The reaction of demethylation mediated by cytochrome P450 (CYP) leads to the equimolar production of demethylated metabolite and formaldehyde. From a 13C-substrate labeled on a carbon of the methyl moiety, (13C)formaldehyde (H13CHO) is liberated. A highly sensitive and specific assay involving the oxidation of H13CHO to 13CO2 by a double-enzymatic-step reaction is reported. The 13CO2 was quantified by the method of reverse isotopic dilution based on gas chromatography-isotope ratio mass spectrometry analysis. The method first involves the limiting step of the CYP-dependent reaction, which is stopped with a mixture of zinc sulfate 5 mM and trichloroacetic acid 100 mM. Then, the transformation of H13CHO to 13CO2 is performed with the formaldehyde (0.2 unit) and the formate (0.2 unit) dehydrogenase NAD-dependent **enzymes**. The recovery of 13CO2 from the incubation mixture was equal to 91.4+-3.0%. The accuracy and the precision of the present method were within 12 and 10%, respectively. The limit of quantification was set to 25 pmol. The performance of the assay was validated on human liver microsomes with five probes: (13C)erythromycin, (1-13C)caffeine, (3-13C)caffeine, (7-13C)caffeine, and (13C2)aminopyrine. This method is useful for the rapid determination of N-demethylase activity of human liver microsomes from methyl-13C-substrates.

L26 ANSWER 10 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:378346 BIOSIS
DOCUMENT NUMBER: PREV200200378346
TITLE: A radiometric assay for glutamine:fructose-6-phosphate amidotransferase.
AUTHOR(S): Broschat, Kay O. [Reprint author]; Gorka, Christine [Reprint author]; Kasten, Thomas P.; Gulve, Eric A. [Reprint author]; Kilpatrick, Brian
CORPORATE SOURCE: Cardiovascular and Metabolic Diseases, Pharmacia Corp., 800 N. Lindbergh Blvd., St. Louis, MO, 63167, USA
SOURCE: Analytical Biochemistry, (June 1, 2002) Vol. 305, No. 1, pp. 10-15. print.
CODEN: ANBCA2. ISSN: 0003-2697.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Jul 2002
Last Updated on STN: 10 Jul 2002

AB Glutamine:fructose-6-phosphate amidotransferase (**GFAT**) catalyzes the first step in the biosynthesis of amino sugars by transferring the amino group from L-glutamine to the acceptor substrate, fructose 6-phosphate, generating the products glucosamine 6-phosphate and glutamic acid. We describe a method for the synthesis and purification of the substrate, fructose 6-phosphate, and methods for a radiometric assay of human **GFAT1** that can be performed in either of two formats: a small disposable-column format and a high-throughput 96-well-plate format. The method performed in the column format can **detect** 1 pmol of glucosamine 6-phosphate, much less than that required by previously

published assays that **measure** GlcN 6-phosphate. The column assay demonstrates a broad linear range with low variability. In both formats, the assay is linear with **time** and **enzyme** concentration and is highly reproducible. This method greatly improves the sensitivity and speed with which **GFAT1** activity can be **measured** and facilitates direct kinetic **measurement** of the transferase activity.

L26 ANSWER 11 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:435353 BIOSIS
DOCUMENT NUMBER: PREV200200435353
TITLE: Development of a fluorescence polarization assay for peptidyl-tRNA hydrolase.
AUTHOR(S): Bonin, Paul D. [Reprint author]; Erickson, Laurence A. [Reprint author]
CORPORATE SOURCE: Pharmacia Corporation, Kalamazoo, MI, 49001, USA
SOURCE: Analytical Biochemistry, (July 1, 2002) Vol. 306, No. 1, pp. 8-16. print.
CODEN: ANBCA2. ISSN: 0003-2697.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Aug 2002
Last Updated on STN: 14 Aug 2002

AB Peptidyl-tRNA hydrolase (Pth) activity ensures the rapid recycling of peptidyl-tRNAs that result from premature termination of translation. Historically, the hydrolyzing activity of Pth has been assayed with radiolabeled N-blocked aminoacyl-tRNAs in assay systems that require the separation of radiolabeled amino acid from the N-blocked aminoacyl-tRNA complex. In the present study, we describe the development of a kinetic fluorescence polarization (FP) assay that enables measurements of Pth activity without the need to separate bound and free tracer. The hydrolyzing activity of Pth was determined by measuring the change in polarization values that resulted from the cleavage of a fluorescently **labeled substrate** (BODIPY-Lys-tRNALys). The data were analyzed using an equation describing first-order dissociation and the results showed that the experimental data correlated well with the theoretical curve. A runs test of the residuals showed that the experimental data did not significantly differ from the first-order model. The assay is adaptable to a multiwell format and is sensitive enough to detect Pth-like activity in bacterial cell lysate. The Pth FP assay provides a homogeneous and kinetic format for measuring Pth activity in vitro.

L26 ANSWER 12 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:427237 BIOSIS
DOCUMENT NUMBER: PREV200100427237
TITLE: Ligands of macrophage scavenger receptor induce cytokine expression via differential modulation of protein **kinase** signaling pathways.
AUTHOR(S): Hsu, Hsien-Yeh [Reprint author]; Chiu, Show-Lan; Wen, Meng-Hsuan; Chen, Kuo-Yen; Hua, Kuo-Feng
CORPORATE SOURCE: Faculty of Medical Technology, Institute of Biotechnology in Medicine, National Yang-Ming University, 155 Li-Nong St., Shih-Pai, Taipei, 112, Taiwan
hyhsu@ym.edu.tw
SOURCE: Journal of Biological Chemistry, (August 3, 2001) Vol. 276, No. 31, pp. 28719-28730. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English

ENTRY DATE: Entered STN: 12 Sep 2001
Last Updated on STN: 22 Feb 2002

AB Our previous works demonstrated that ligands of macrophage scavenger receptor (MSR) induce protein **kinases** (PKs) including protein-tyrosine **kinase** (PTK) and up-regulate **urokinase** -type plasminogen activator expression (Hsu, H. Y., Hajjar, D. P., Khan, K. M., and Falcone, D. J. (1998) J. Biol. Chem. 273, 1240-1246). To continue to investigate MSR ligand-mediated signal transductions, we focus on ligands, oxidized low density lipoprotein (OxLDL), and fucoidan induction of the cytokines tumor necrosis factor-alpha (TNF) and interleukin 1beta (IL-1). In brief, in murine macrophages J774A.1, OxLDL and fucoidan up-regulate TNF production; additionally, fucoidan but not OxLDL induces IL-1 secretion, prointerleukin 1 (proIL-1, precursor of IL-1) protein, and proIL-1 message. Simultaneously, fucoidan stimulates activity of interleukin 1-converting **enzyme**. We further investigate the molecular mechanism by which ligand **binding** -induced PK-mediated mitogen-activated protein **kinase** (MAPK) in regulation of expression of proIL-1 and IL-1. Specifically, fucoidan stimulates activity of p21-activated **kinase** (PAK) and of the MAPKs extracellular signal-regulated **kinase** (ERK), c-Jun NH2-terminal **kinase** (JNK), and **p38**. Combined with PK inhibitors and genetic mutants of Rac1 and JNK in PK activity assays, Western blotting analyses, and IL-1 **enzyme**-linked immunosorbent assay, the role of individual PKs in the regulation of proIL-1/IL-1 was extensively dissected. Moreover, tyrosine phosphorylation of pp60Src as well as association between pp60Src and Hsp90 play important roles in fucoidan-induced proIL-1 expression. We are the first to establish two fucoidan-mediated signaling pathways: PTK(Src)/Rac1/PAK/JNK and PTK(Src)/Rac1/PAK/**p38**, but not PTK/phospholipase C-gamma1/PKC/MEK1/ERK, playing critical roles in proIL-1/IL-1 regulation. Our current results indicate and suggest a model for MSR ligands differentially modulating specific PH signal transduction pathways, which regulate atherogenesis-related inflammatory cytokines TNF and IL-1.

L26 ANSWER 13 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:334377 BIOSIS
DOCUMENT NUMBER: PREV200100334377
TITLE: Determination of the midpoint potential of the FAD and FMN flavin cofactors and of the 3Fe-4S cluster of glutamate synthase.
AUTHOR(S): Ravasio, Sergio; Curti, Bruno; Vanoni, Maria A. [Reprint author]
CORPORATE SOURCE: Dipartimento di Fisiologia e Biochimica Generali, Universita degli Studi di Milano, Via Celoria 26, 20133, Milano, Italy
mav@mailserver.unimi.it
SOURCE: Biochemistry, (May 8, 2001) Vol. 40, No. 18, pp. 5533-5541. print.
CODEN: BICHAW. ISSN: 0006-2960.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 18 Jul 2001
Last Updated on STN: 19 Feb 2002

AB Glutamate synthase is a complex iron-sulfur flavoprotein that catalyzes the reductive transfer of the L-glutamine amide group to C(2) of 2-oxoglutarate, forming two molecules of L-glutamate. The bacterial **enzyme** is an alphabeta protomer, which contains one FAD (on the beta subunit, apprx50 kDa), one FMN (on the alpha subunit, kappa150 kDa), and three different Fe-S clusters (one 3Fe-4S center on the alpha subunit and two 4Fe-4S clusters at an unknown location). To address the problem

of the intramolecular electron pathway, we have **measured** the midpoint potential values of the flavin cofactors and of the 3Fe-4S cluster of glutamate synthase in the isolated alpha and beta subunits and in the alphabeta **holoenzyme**. No **detectable** amounts of flavin semiquinones were observed during reductive titrations of the **enzyme**, indicating that the midpoint potential value of each flavinox/flavinsq **couple** is, in all cases, significantly more negative than that of the corresponding flavinsq/flavinhq **couple**. Association of the two subunits to form the alphabeta protomer does not alter significantly the midpoint potential value of the FMN cofactor and of the 3Fe-4S cluster (approximately -240 and -270 mV, respectively), but it makes that of FAD some 40 mV less negative (approximately -340 mV for the beta subunit and -300 mV for FAD **bound** to the **holoenzyme**). **Binding** of the nonreducible NADP+ analogue, 3-aminopyridine adenine dinucleotide phosphate, made the **measured** midpoint potential value of the FAD cofactor approximately 30-40 mV less negative in the isolated beta subunit, but had no effect on the redox properties of the alphabeta **holoenzyme**. This result correlates with the formation of a stable charge-transfer complex between the reduced flavin and the oxidized pyridine nucleotide in the isolated beta subunit, but not in the alphabeta **holoenzyme**. **Binding** of L-**methionine** sulfone, a glutamine analogue, had no significant effect on the redox properties of the **enzyme** cofactors. On the contrary, 2-oxoglutarate made the **measured** midpoint potential value of the 3Fe-4S cluster approximately 20 mV more negative in the isolated alpha subunit, but up to 100 mV less negative in the alphabeta **holoenzyme** as compared to the values of the corresponding free **enzyme** forms. These findings are consistent with electron transfer from the entry site (FAD) to the exit site (FMN) through the 3Fe-4S center of the **enzyme** and the involvement of at least one of the two low-potential 4Fe-4S centers, which are present in the glutamate synthase **holoenzyme**, but not in the isolated subunits. Furthermore, the data demonstrate a specific role of 2-oxoglutarate in promoting electron transfer from FAD to the 3Fe-4S cluster of the glutamate synthase **holoenzyme**. The modulatory role of 2-oxoglutarate is indeed consistent with the recently **determined** three-dimensional structure of the glutamate synthase alpha subunit, in which several polypeptide stretches are suitably positioned to mediate communication between substrate **binding** sites and the **enzyme** redox centers (FMN and the 3Fe-4S cluster) to tightly **control** and coordinate the individual reaction steps (**Binda**, C., et al. (2000) Structure 8, 1299-1308).

L26 ANSWER 14 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:258307 BIOSIS
DOCUMENT NUMBER: PREV200100258307
TITLE: Endotoxin (LPS) and interferon gamma induced dendritic cell apoptosis is mediated by nitric oxide production and Caspase-3.
AUTHOR(S): Stanford, Ala [Reprint author]; Chen, Yue; Hoffman, Rosemary; Ford, Henri R.
CORPORATE SOURCE: Children's Hospital of Pittsburgh, 3705 Fifth Avenue, Pittsburgh, PA, 15221, USA
SOURCE: FASEB Journal, (March 8, 2001) Vol. 15, No. 5, pp. A939. print.
Meeting Info.: Annual Meeting of the Federation of American Societies for Experimental Biology on Experimental Biology 2001. Orlando, Florida, USA. March 31-April 04, 2001.
CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 30 May 2001
Last Updated on STN: 19 Feb 2002

AB Purpose: Dendritic cells (DC) play a crucial role in amplifying the immune response to combat invading pathogens and prevent tumorigenesis by stimulating both T and B-lymphocytes. Thus, dysregulation or death of DCs can predispose to infection, tumor implantation or alternatively, autoimmune disease. We have previously shown that the nitric oxide (NO) donor SNAP (s-nitroso-N-acetyl penicillamine) induces DC apoptosis in vitro. In this study we examined the effect of endogenous NO production on DCs stimulated with lipopolysaccharide (endotoxin or LPS) and interferon-gamma (IFN-gamma). Methods: DC2.4 cells, a bone marrow derived DC line (Dana Farber Cancer Institute), were incubated with 100u/ml IFN-gamma and 1ug/ml LPS. Flow cytometry with Annexin V and propidium iodide staining was used to **measure** apoptosis and necrosis. Supernatant nitrite (NO2-) levels were **measured** using the Greiss reaction. DC2.4 were also co-incubated with L-NIL, a specific inhibitor of inducible **nitric oxide synthase**. **Enzyme activity assay** was used to **measure** effector Caspase-3 activity. Results: LPS and IFN-gamma induced DC2.4 apoptosis and necrosis (Media at 24 hours 21.260 +/- 1.8 % vs. LPS + IFN-gamma 75.5 +/- 3.4%). Addition of 1mM L-NIL suppressed LPS and IFN-gamma induced cell death (75.5 +/- 3.4 % to 48.97 +/- 3.5 %) and concurrently increased live cell number L-NIL also decreased NO2-production (56.4 uM to 1.92 uM), which correlated with the decrease in DC2.4 death. Caspase-3 activity was significantly increased at 4h following exposure to LPS and IFN-gamma compared to the negative **control** (0.01 OD/ug protein/hr vs. 0.31 OD/ug protein/hr). Conclusion: LPS and IFN-gamma induced DC2.4 apoptosis may be dependent on nitric oxide production. Interestingly, with the addition of L-NIL the percentage of apoptosis does not decrease to baseline levels suggesting that other factors may also be involved. Therefore elucidation of cell death pathways by these inducers may provide valuable information in developing strategies to protect the host dendritic cells.

L26 ANSWER 15 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:344421 BIOSIS
DOCUMENT NUMBER: PREV200100344421
TITLE: Characterization of cyclodextrin glycosyltransferase from Bacillus firmus strain no. 37.
AUTHOR(S): Matioli, Graciette; Zanin, Gisella M.; De Moraes, Flavio F. [Reprint author]
CORPORATE SOURCE: Chemical Engineering Department, State University of Maringa, Av. Colombo, 5790, BL D-90, 87020-900, Maringa, PR, Brazil
flavio@maringa.com.br
SOURCE: Applied Biochemistry and Biotechnology, (Spring, 2001) Vol. 91-93, pp. 643-654. print.
CODEN: ABIBDL. ISSN: 0273-2289.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 25 Jul 2001
Last Updated on STN: 19 Feb 2002

AB The **enzyme** cyclodextrin glycosyltransferase (**CGTase**), EC 2.4.1.19, which produces cyclodextrins (CDs) from starch, was obtained from Bacillus firmus strain no. 37 isolated from Brazilian soil and characterized in the soluble form using as substrate 100 g/L of maltodextrin in 0.05 M Tris-HCl buffer, 5 mM CaCl2, and appropriate buffers. Enzymatic activity and its activation energy were

determined as a function of temperature and pH. The activation energy for the production of beta- and gamma-CD was 7.5 and 9.9 kcal/mol, respectively. The energy of deactivation was 39 kcal/mol. The **enzyme** showed little thermal deactivation in the temperature range of 35-60degreeC, and Arrhenius-type equations were obtained for calculating the activity, deactivation, and half-life as a function of temperature. The molecular weight of the **enzyme** was **determined** by sodium dodecyl sulfate polyacrylamide gel electrophoresis, giving 77.6 kDa. Results for **CGTase** activity as a function of temperature gave maximal activity for the production of beta-CD at 65degreeC, pH 6.0, and 71.5 mmol of beta-CD/(min cntdot mg of protein), whereas for gamma-CD it was 9.1 mmol of gamma-CD/(min cntdot mg of protein) at 70degreeC and pH 8.0. For long contact **times**, the best use of the enzymatic activity occurs at 60degreeC or at a lower temperature, and the reaction pH may be selected to increase the yield of a desired CD.

L26 ANSWER 16 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2002:163635 BIOSIS
DOCUMENT NUMBER: PREV200200163635
TITLE: Agrobacterium-mediated transformation of Vicia faba.
AUTHOR(S): Boettinger, Petra; Steinmetz, Anke; Schieder, Otto; Pickardt, Thomas [Reprint author]
CORPORATE SOURCE: Institute for Applied Genetics, Free University of Berlin, Albrecht-Thaer-Weg 6, 14195, Berlin, Germany petrab@zedat.fu-berlin.de; pickardt@zedat.fu-berlin.de
SOURCE: Molecular Breeding, (October, 2001) Vol. 8, No. 3, pp. 243-254. print.
ISSN: 1380-3743.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Mar 2002
Last Updated on STN: 5 Mar 2002

AB Among the major grain legume crops, Vicia faba belongs to those where the production of transgenic plants has not yet convincingly been reported. We have produced stably transformed lines of faba bean with an Agrobacterium tumefaciens-mediated gene transfer system. Stem segments from aseptically germinated seedlings were inoculated with A. tumefaciens strains EHA101 or EHA105, carrying binary vectors conferring (1) uidA, (2) a mutant lysC gene, coding for a bacterial aspartate **kinase** insensitive to feedback **control** by threonine, and (3) the coding sequence for a **methionine**-rich sunflower 2S-albumin, each in combination with nptII as selectable marker. Kanamycin-resistant calluses were obtained on callus initiation medium at a frequency of 10-30%. Shoot regeneration was achieved on thidiazuron containing medium in a second culture step. A subsequent transfer of shoots to BA-containing medium was necessary for stem elongation and leaf development. Shoots were rooted or grafted onto young seedlings in vitro and mature plants were recovered. Molecular analysis confirmed the integration of the transgenes into the plant genome. Inheritance and expression of the foreign genes was demonstrated by Southern blot, PCR, western analysis and **enzyme activity assays**. Although at present the system is **time**-consuming and of relatively low efficiency, it represents a feasible approach for the production of genetically engineered faba beans.

L26 ANSWER 17 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:301907 BIOSIS
DOCUMENT NUMBER: PREV200100301907
TITLE: Purification and properties of cyclodextrin glucanotransferase synthesizing 2-O-alpha-D-glucopyranosyl

L-ascorbic acid from Paenibacillus sp. JB-13.
AUTHOR(S): Bae, Kyung-Mi; Kim, Sung-Koo; Kong, In-Soo; Jun, Hong-Ki [Reprint author]
CORPORATE SOURCE: Division of Biological Sciences, Pusan National University, Pusan, 609-735, South Korea
hkjun@hyowon.cc.pusan.ac.kr
SOURCE: Journal of Microbiology and Biotechnology, (April, 2001) Vol. 11, No. 2, pp. 242-250. print.
ISSN: 1017-7825.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Jun 2001
Last Updated on STN: 19 Feb 2002

AB A Gram-positive bacterium (strain JB-13) that was isolated from soil as a producer of cyclodextrin glucanotransferase (**CGTase**) (EC 2.4.1.19) was identified as Paenibacillus sp. JB-13. This **CGTase** could catalyze the transglucosylation reaction from soluble starch to L-ascorbic acid (AA). A main product formed by this **enzyme** with alpha-glucosidase was identified as 2-O-alpha-D-glucopyranosyl L-ascorbic acid (AA-2G) by the HPLC profile and the elemental analysis. **CGTase** was purified to homogeneity using ammonium sulfate fractionation, ion-exchange chromatography on DEAE-Sephadex A-50, and gel chromatography on Sephacryl S-200HR. The molecular weight was **determined** to be 66,000 by both gel chromatography and SDS-PAGE. The isoelectric point of the purified **enzyme** was 5.3. The optimum pH and temperature was pH 7.0 and 45degreeC, respectively. The **enzyme** was stable in the range of pH 6- 9 and at temperatures of 75degreeC or less in the presence of 15 mM CaCl2. Hg2+, Mn2+, Ag+, and Cu2+ all strongly inhibited the **enzyme**'s activity.

L26 ANSWER 18 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:339966 BIOSIS
DOCUMENT NUMBER: PREV200000339966
TITLE: Inhibition by extracellular cAMP of phorbol 12-myristate 13-acetate-induced prostaglandin H synthase-2 expression in human pulmonary microvascular endothelial cells: Involvement of an ecto-protein **kinase** A activity.
AUTHOR(S): Elalamy, Ismail; Said, Fatima Ait; Singer, Monique; Couetil, Jean-Paul; Hatmi, Mohamed [Reprint author]
CORPORATE SOURCE: Unite de Pharmacologie Cellulaire, Unite Associee Institut Pasteur-INSERM U 485, 25 Rue du Dr. Roux, 75724, Paris Cedex 15, France
SOURCE: Journal of Biological Chemistry, (May 5, 2000) Vol. 275, No. 18, pp. 13662-13667. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Aug 2000
Last Updated on STN: 7 Jan 2002

AB Exposure of human pulmonary microvascular endothelial cells (HPMECs) to phorbol 12-myristate 13-acetate (PMA) leads to the increase of prostaglandin H synthase (PGHS)-2 protein levels. Under same conditions and according to its constitutive nature, no significant variation of PGHS-1 protein was noted. The elevation of the intracellular cAMP rate is known to enhance PGHS-2 levels through a protein **kinase** A pathway in various cells. To **determine** whether the extracellular cAMP also regulates the inducible expression of PGHS, cultured HPMECs were exposed to cAMP alone or in combination with PMA. The PMA-induced PGHS-2 protein was attenuated by the extracellular cAMP. In addition, PGHS-2 activity evaluated through 6-keto-PGF1alpha

generation, which was enhanced by PMA was inhibited by extracellular cAMP. Furthermore, in HPMEC medium, PMA-induced PGHS-2 expression was accompanied by the generation of a transferable activity (TA) able to abolish platelet aggregation. This resulting TA was dependent from PGHS-2 pathway, because NS-398, a selective inhibitor of PGHS-2, suppressed its production. The inhibitory TA released by treated HPMECs was also prevented by extracellular cAMP. The specific protein **kinase** A (PKA) inhibitor blocked the extracellular cAMP effect on both PMA-induced 6-keto-PGF1alpha synthesis and inhibitory TA generation, suggesting the involvement of PKA signaling at the outer surface of HPMECs. Accordingly, we established, in phosphorylation experiments, the presence of an endothelial ecto-protein **kinase** activity, able to phosphorylate the synthetic substrate kemptide in a cAMP-dependent mode. Reverse transcription-polymerase chain reaction analysis showed that PMA-induced PGHS-2 mRNA was markedly reduced by extracellular cAMP. Together, these findings provide the first experimental evidence that extracellular cAMP is able to reduce HPMEC PGHS-2 expression in terms of mRNA, protein, and **enzyme** activity through an ecto-PKA pathway. In addition, they outline the potential role of endothelial PGHS-2 in the limitation of platelet activation during inflammatory processes.

L26 ANSWER 19 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:329359 BIOSIS
DOCUMENT NUMBER: PREV200000329359
TITLE: Divergence in regulation of **nitric-oxide synthase** and its cofactor tetrahydrobiopterin by tumor necrosis factor-alpha: Ceramide potentiates nitric oxide synthesis without affecting GTP cyclohydrolase I activity.
AUTHOR(S): Vann, Lewis R.; Twitty, Sharon; Spiegel, Sarah; Milstien, Sheldon [Reprint author]
CORPORATE SOURCE: LCMR, NIMH, National Institutes of Health, Bldg. 36, Rm. 2A-11, Bethesda, MD, 20892, USA
SOURCE: Journal of Biological Chemistry, (May 5, 2000) Vol. 275, No. 18, pp. 13275-13281. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Aug 2000
Last Updated on STN: 7 Jan 2002

AB Synthesis of 6(R)-5,6,7,8-tetrahydrobiopterin (BH4), a required cofactor for inducible **nitric-oxide synthase** (iNOS) activity, is usually coordinately regulated with iNOS expression. In C6 glioma cells, tumor necrosis factor-alpha (TNF-alpha) concomitantly potentiated the stimulation of nitric oxide (NO) and BH4 production induced by IFN-gamma and interleukin-1beta. Expression of both iNOS and GTP cyclohydrolase I (GTPCH), the rate-limiting **enzyme** in the BH4 biosynthetic pathway, was also markedly increased, as were their activities and protein levels. Ceramide, a sphingolipid metabolite, may mediate some of the actions of TNF-alpha. Indeed, we found that bacterial sphingomyelinase, which hydrolyzes sphingomyelin and increases endogenous ceramide, or the cell permeable ceramide analogue, C2-ceramide, but not C2-dihydroceramide (N-acetylsphinganine), significantly mimicked the effects of TNF-alpha on NO production and iNOS expression and activity in C6 cells. Surprisingly, although TNF-alpha increased BH4 synthesis and GTPCH activity, neither BH4 nor GTPCH expression was affected by C2-ceramide or sphingomyelinase in IFN-gamma- and interleukin-1beta-stimulated cells. It is likely that increased BH4 levels results from increased GTPCH protein and activity in vivo rather than from reduced turnover of BH4, because the GTPCH inhibitor, 2,4-diamino-6-

hydroxypyrimidine, blocked cytokine-stimulated BH4 accumulation. Moreover, expression of the GTPCH feedback regulatory protein, which if decreased might increase GTPCH activity, was not affected by TNF-alpha or ceramide. Treatment with the antioxidant pyrrolidine dithiocarbamate, which is known to inhibit NF-kappaB and sphingomyelinase in C6 cells, or with the peptide SN-50, which blocks translocation of NF-kappaB to the nucleus, inhibited TNF-alpha-dependent iNOS mRNA expression without affecting GTPCH mRNA levels. This is the first demonstration that cytokine-stimulated iNOS and GTPCH expression, and therefore NO and BH4 biosynthesis, may be regulated by discrete pathways. As BH4 is also a cofactor for the aromatic amino acid hydroxylases, discovery of distinct mechanisms for regulation of BH4 and NO has important implications for its specific functions.

L26 ANSWER 20 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:329355 BIOSIS
DOCUMENT NUMBER: PREV200000329355
TITLE: P2Y receptor-mediated inhibition of tumor necrosis factor alpha-stimulated stress-activated protein **kinase** activity in EAhy926 endothelial cells.
AUTHOR(S): Paul, Andrew; Torrie, Lindsay J.; McLaren, Gerald J.; Kennedy, Charles; Gould, Gwyn W.; Plevin, Robin [Reprint author]
CORPORATE SOURCE: Department of Physiology and Pharmacology, University of Strathclyde, Strathclyde Institute for Biomedical Sciences, 27 Taylor Street, Glasgow, G4 0NR, UK
SOURCE: Journal of Biological Chemistry, (May 5, 2000) Vol. 275, No. 18, pp. 13243-13249. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Aug 2000
Last Updated on STN: 7 Jan 2002

AB In the EAhy926 endothelial cell line, UTP, ATP, and forskolin, but not UDP and epidermal growth factor, inhibited tumor necrosis factor alpha (TNFalpha)- and sorbitol stimulation of the stress-activated protein **kinases**, JNK, and **p38** mitogen-activated protein (MAP) **kinase**, and MAPKAP **kinase**-2, the downstream target of **p38** MAP **kinase**. In NCT2544 keratinocytes, UTP and a proteinase-activated receptor-2 agonist caused similar inhibition, but in 13121N1 cells, transfected with the human P2Y2 or P2Y4 receptor, UTP stimulated JNK and **p38** MAP **kinase** activities. This suggests that the effects mediated by P2Y receptors are cell-specific. The inhibitory effects of UTP were not due to induction of MAP **kinase** phosphatase-1, but were manifest upstream in the pathway at the level of MEK-4. The inhibitory effect of UTP was insensitive to the MEK-1 inhibitor PD 098059, changes in intracellular Ca2+ levels, or pertussis toxin. Acute phorbol 12-myristate 13-acetate pretreatment also inhibited TNFalpha-stimulated SAP **kinase** activity, while chronic pretreatment reversed the effects of UTP. Furthermore, the protein **kinase** C inhibitors Ro318220 and Go6983 reversed the inhibitory action of UTP, but GF109203X was ineffective. These results indicate a novel mechanism of cross-talk regulation between P2Y receptors and TNFalpha-stimulated SAP **kinase** pathways in endothelial cells, mediated by Ca2+-independent isoforms of protein **kinase** C.

L26 ANSWER 21 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:152046 BIOSIS
DOCUMENT NUMBER: PREV200100152046
TITLE: Adsorption of inulinases in ion-exchange columns.

AUTHOR(S): Silva, F.-R. C.; Santana, C. C. [Reprint author]
CORPORATE SOURCE: School of Chemical Engineering, Universidade Estadual de Campinas, UNICAMP, CEP13083-970, Cammpinas, SP, Brazil santana@feq.unicamp.br
SOURCE: Applied Biochemistry and Biotechnology, (Spring, 2000) Vol. 84-86, pp. 1063-1078. print.
CODEN: ABIBDL. ISSN: 0273-2289.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 28 Mar 2001
Last Updated on STN: 15 Feb 2002

AB The use of adsorption columns packed with **ion-exchange resins** for recovering, concentrating and purifying proteins is now widespread. The present work consists of a study on the dynamic behavior of adsorption columns that uses two kinds of adsorbents: a cationic and an anionic resin. A frontal analysis of the columns was performed with experimental data obtained from Fructozyme, a mixture of inulinase **enzymes**. The parameters of a Langmuir type of isotherm and adsorption kinetics were obtained from experimental tests in a batch system. A numerical technique based on orthogonal collocation and a fourth-order Runge-Kutta method was coupled with a nonlinear optimization method to predict the coefficients of the rate equations, which are fundamental for scale-up purposes.

L26 ANSWER 22 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:503532 BIOSIS
DOCUMENT NUMBER: PREV200000503532
TITLE: **Detection** and substrate selectivity of new microbial D-amino acid oxidases.
AUTHOR(S): Gabler, M.; Hensel, M.; Fischer, L. [Reprint author]
CORPORATE SOURCE: Division of Biotechnology, Institute of Food Technology, University of Hohenheim, Emil-Wolff-Str. 14, D-70599, Stuttgart, Germany
SOURCE: Enzyme and Microbial Technology, (November 1, 2000) Vol. 27, No. 8, pp. 605-611. print.
CODEN: EMTED2. ISSN: 0141-0229.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Nov 2000
Last Updated on STN: 11 Jan 2002

AB In order to screen for new microbial D-amino acid oxidase activities a selective and sensitive peroxidase/o-dianisidine assay, **detecting** the formation of hydrogen peroxide was developed. Catalase, which coexists with oxidases in the peroxisomes or the microsomes and, which competes with peroxidase for hydrogen peroxide, was completely inhibited by o-dianisidine up to a catalase activity of 500 nkat ml-1. Thus, using the peroxidase/o-dianisidine assay and employing crude extracts of microoganisms in a microplate reader, a **detection** sensitivity for oxidase activity of 0.6 nkat ml-1 was obtained. Wild type colonies which were grown on a selective medium containing D-alanine as carbon, energy and nitrogen source were examined for D-amino acid oxidase activity by the peroxidase/o-dianisidine assay. The oxidase positive colonies possessing an apparent oxidase activity > 2 nkat g dry biomass-1 were isolated. Among them three new D-amino acid oxidase-producers were found and identified as Fusarium oxysporum, Verticilium lutealbum and Candida parapsilosis. The best new D-amino oxidase producer was the fungus F. oxysporum with a D-amino acid oxidase activity of about 900 nkat g dry biomass-1 or 21 nkat mg protein-1. With regard to the use as a biocatalytic tool in biotechnology the substrate specificities of the three new D-amino acid oxidases were compared with those of the known

D-amino acid oxidases from Trigonopsis variabilis, Rhodotorula gracilis and pig kidney under the same conditions. All six D-amino acid oxidases accepted the D-enantiomers of alanine, valine, leucine, proline, phenylalanine, serine and glutamine as substrates and, except for the D-amino acid oxidase from V. luteoalbum, D-tryptophane, D-tyrosine, D-arginine and D-histidine were accepted as well. The relative highest activities (>95%) were **measured** versus D-alanine (C. parapsilosis, F. oxysporum, T. variabilis), D-**methionine** (V. luteoalbum, R. gracilis), D-valine (T. variabilis, R. gracilis) and D-proline (pig kidney). The D-amino oxidases from F. oxysporum and V. luteoalbum were able to react with the industrially important substrate cephalosporin C although the D-amino acid oxidase from T. variabilis was at least about 20-fold more active with this substrate. As the results of our studies, a reliable oxidase assay was developed, allowing high throughput screening in a microplate reader. Furthermore, three new microbial D-amino acid oxidase-producers with interesting broad substrate specificities were introduced in the field of biotechnology.

L26 ANSWER 23 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:233637 BIOSIS
DOCUMENT NUMBER: PREV200000233637
TITLE: Refolding and purification of Zymomonas mobilis levansucrase produced as inclusion bodies in fed-batch culture of recombinant Escherichia coli.
AUTHOR(S): Sunitha, Kandula; Chung, Bong Hyun [Reprint author]; Jang, Ki-Hyo; Song, Ki-Bang; Kim, Chul Ho; Rhee, Sang-Ki
CORPORATE SOURCE: Korea Research Institute of Bioscience and Biotechnology, Yusong, Taejon, 305-600, South Korea
SOURCE: Protein Expression and Purification, (April, 2000) Vol. 18, No. 3, pp. 388-393. print.
CODEN: PEXPEJ. ISSN: 1046-5928.
DOCUMENT TYPE: Article
LANGUAGE: English
OTHER SOURCE: Genbank-L34331; Genbank-U91484
ENTRY DATE: Entered STN: 7 Jun 2000
Last Updated on STN: 5 Jan 2002

AB Zymomonas mobilis levansucrase was overproduced by the fed-batch culture of recombinant Escherichia coli harboring a novel expression system that is constitutively expressed by the promoter from the Rahnella aquatilis levansucrase gene. Most of the levansucrase was produced as inclusion bodies in the bacterial cytoplasm, accounting for approximately 20% of the total cellular protein. Refolding after complete denaturation by high concentrations of urea or guanidine hydrochloride was not successful, resulting in large amounts of insoluble aggregates. During the development of the refolding method, it was found that direct solubilization of the inclusion bodies with Triton X-100 reactivated the **enzyme**, with a considerable refolding efficiency. About 65% of inclusion body levansucrase was refolded into active levansucrase in the renaturation buffer containing 4% (v/v) Triton X-100. The in vitro refolded **enzyme** was purified to 95% purity by single-step DEAE-Sepharose ion exchange chromatography. Triton X-100 was removed by this ion exchange chromatography.

L26 ANSWER 24 OF 51 MEDLINE on STN
ACCESSION NUMBER: 2001257514 MEDLINE
DOCUMENT NUMBER: 21087730 PubMed ID: 11219738
TITLE: Screen-printed amperometric microcell for proline iminopeptidase **enzyme activity assay.**
AUTHOR: Nagy G; Gyurcsanyi R E; Cristalli C A; Neuman M R; Lindner

E
CORPORATE SOURCE: Joint Graduate Program in Biomedical Engineering, The University of Memphis and University of Tennessee Health Science Center, Herff College of Engineering, 38152-6582, USA.
SOURCE: BIOSENSORS AND BIOELECTRONICS, (2000 Aug) 15 (5-6) 265-72. Journal code: 9001289. ISSN: 0956-5663.
PUB. COUNTRY: England: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010521
Last Updated on STN: 20010521
Entered Medline: 20010517

AB A microfabricated amperometric microcell was designed and used for the determination of proline iminopeptidase (PIP) **enzyme** activity in 2-10-microl samples. The **measurements** were made in the range of 10.3-841.5 mU/ml **enzyme** activities. The sensitivity of the determinations was between - 0.0195 and - 0.0203 microA ml/mU per min. The coefficient of variation of the **determined** values ranged between 2.8 (at 561.2 mU/ml) and 24.1% (at 10.3 mU/ml). The microcell was manufactured on an alumina substrate using screen-printed graphite working and Ag/AgCl reference electrodes. Elevated PIP activity in the vaginal **fluid** is a biochemical indicator of bacterial vaginosis. The method is appropriate to differentiate between normal (66+/-145 mU/ml) and elevated, diseased (704+/-145 mU/ml), values.

L26 ANSWER 25 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:26854 BIOSIS
DOCUMENT NUMBER: PREV200000026854
TITLE: Functional characterization of human methylenetetrahydrofolate reductase in Saccharomyces cerevisiae.
AUTHOR(S): Shan, Xiaoyin; Wang, Liqun; Hoffmaster, Roselle; Kruger, Warren D. [Reprint author]
CORPORATE SOURCE: Division of Population Science, Fox Chase Cancer Center, 7701 Burholme Ave., Philadelphia, PA, 19111, USA
SOURCE: Journal of Biological Chemistry, (Nov. 12, 1999) Vol. 274, No. 46, pp. 32613-32618. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 13 Jan 2000
Last Updated on STN: 31 Dec 2001

AB Human methylenetetrahydrofolate reductase (MTHFR, EC 1.5.1.20) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. 5-Methyltetrahydrofolate is a major methyl donor in the remethylation of homocysteine to **methionine**. Impaired MTHFR can cause high levels of homocysteine in plasma, which is an independent risk factor for vascular disease and neural tube defects. We have functionally characterized wild-type and several mutant alleles of human MTHFR in yeast, Saccharomyces cerevisiae. We have shown that yeast MET11 is a functional homologue of human MTHFR. Expression of the human MTHFR cDNA in a yeast strain deleted for MET11 can restore the strain's MTHFR activity in vitro and complement its **methionine** auxotrophic phenotype in vivo. To understand the domain structure of human MTHFR, we have truncated the C terminus (50%) of the protein and demonstrated that expressing an N-terminal human MTHFR in met11- yeast cells rescues the growth phenotype, indicating that this region contains

the catalytic domain of the **enzyme**. However, the truncation leads to the reduced protein levels, suggesting that the C terminus may be important for protein stabilization. We have also functionally characterized four missense mutations identified from patients with severe MTHFR deficiency and two common missense polymorphisms found at high frequency in the general population. Three of the four missense mutations are unable to complement the auxotrophic phenotype of met11- yeast cells and show less than 7% **enzyme** activity of the wild type in vitro. Both of the two common polymorphisms are able to complement the growth phenotype, although one exhibited thermolabile **enzyme** activity in vitro. These results shall be useful for the functional characterization of MTHFR mutations and analysis structure/function relationship of the **enzyme**.

L26 ANSWER 26 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:482663 BIOSIS
DOCUMENT NUMBER: PREV199900482663
TITLE: Tetrahydrobiopterin-dependent inhibition of superoxide generation from neuronal **nitric oxide synthase**.
AUTHOR(S): Vasquez-Vivar, Jeannette; Hogg, Neil; Martasek, Pavel; Karoui, Hakim; Pritchard, Kirkwood A., Jr.; Kalyanaraman, Balarama [Reprint author]
CORPORATE SOURCE: Biophysics Research Institute, 8701 Watertown Plank Rd., Milwaukee, WI, 53226, USA
SOURCE: Journal of Biological Chemistry, (Sept. 17, 1999) Vol. 274, No. 38, pp. 26736-26742. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Nov 1999
Last Updated on STN: 16 Nov 1999

AB The binding of calcium/calmodulin stimulates electron transfer between the reductase and oxygenase domains of neuronal **nitric oxide synthase** (nNOS). Here, we demonstrate using electron spin resonance spin-trapping with 5-diethoxyphosphoryl-5-methyl-1-pyrroline N-oxide that pterin-free nNOS generates superoxide from the reductase and the oxygenase domain by a calcium/calmodulin-dependent mechanism. Tetrahydrobiopterin (BH4) diminishes the formation of superoxide by a mechanism that does not cause inhibition of NADPH consumption. In contrast, BH4 analogs 7,8-dihydrobiopterin and sepiapterin do not affect superoxide yields. L-Arginine alone inhibits the generation of superoxide by nNOS but not by C331A-nNOS mutant that has a low affinity for L-arginine. A greater decrease in superoxide yields is observed when nNOS is preincubated with L-arginine. This effect is in accordance with the slow binding rates of L-arginine to NOS in the absence of BH4. L-Arginine alone or in combination withBH4 decreases the rates of NADPH consumption. The effect of L-arginine on superoxide yields, however, was less dramatic than that caused by BH4 as much higher concentrations of L-arginine are necessary to attain the same inhibition. In combination, L-arginine and BH4 inhibit the formation of superoxide generation and stimulate the formation of L-citrulline. We conclude that, in contrast to L-arginine, BH4 does not inhibit the generation of superoxide by **controlling** electron transfer through the **enzyme** but by stimulating the formation of the heme-peroxo species.

L26 ANSWER 27 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:442974 BIOSIS
DOCUMENT NUMBER: PREV199900442974
TITLE: Truncation of the C terminus of the rat brain Na+-Ca2+

exchanger RBE-1 (NCX1.4) impairs surface expression of the protein.
AUTHOR(S): Kasir, Judith; Ren, Xiaoyan; Furman, Ian; Rahamimoff, Hannah [Reprint author]
CORPORATE SOURCE: Department of Biochemistry, Hebrew University Hadassah Medical School Jerusalem, 91120, Jerusalem, Israel
SOURCE: Journal of Biological Chemistry, (Aug. 27, 1999) Vol. 274, No. 35, pp. 24873-24880. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 26 Oct 1999
Last Updated on STN: 26 Oct 1999

AB The C terminus of the rat brain Na+-Ca2+ exchanger (RBE-1; NCX1.4) (amino acids 875-903) is modeled to contain the last transmembrane alpha helix (amino acids 875-894) and an intracellular extramembraneous tail of 9 amino acids (895-903). Truncation of the last 9 C-terminal amino acids, Glu-895 to **stop**, did not significantly impair functional expression in HeLa or HEK 293 cells. Truncation, however, of 10 amino acids (Leu-894 to **stop**; mutant C10) reduced Na+ gradient-dependent Ca2+ uptake to 35-39% relative to the wild type parent exchanger, and further truncation of 13 or more amino acids resulted in expression of trace amounts of transport activity. Western analysis indicated that Na+-Ca2+ exchanger protein was produced whether transfection was carried out with functional or non-functional mutants. Immunofluorescence studies of HEK 293 cells expressing N-Flag epitope-tagged wild type and mutant Na+-Ca2+ exchangers revealed that transport activity in whole cells correlated with surface expression. All cells expressing the wild type exchanger or C9 exhibited surface expression of the protein. Only 39% of the cells expressing C10 exhibited surface expression, and none was **detected** in cells transfected with non-functional mutants C13 and C29. Since functional and non-functional mutants were glycosylated, the C terminus is not mandatory to translocation into the endoplasmic reticulum (ER). Endoglycosidase H digestion of (35S)**methionine**-labeled protein derived from wild type Na+-Ca2+ exchanger and from C10 indicated that resistance to the digestion was acquired after 1 and 5 h of chase, respectively. C29 did not acquire **detectable** resistance to endoglycosidase H digestion even after 10 h of chase. Taken together, these results suggest that the "cellular quality **control** machinery" can tolerate the structural change introduced by truncation of the C terminus up to Ser-893 albeit with reduced rate of ERfwdarwGolgi transfer and reduced surface expression of the truncated protein. Further truncation of C-terminal amino acids leads to retention of the truncated protein in the ER, no transfer to the Golgi, and no surface expression.

L26 ANSWER 28 OF 51 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2000154941 MEDLINE
DOCUMENT NUMBER: 20154941 PubMed ID: 10690351
TITLE: Estrogen receptor-alpha gene transfer into bovine aortic endothelial cells induces eNOS gene expression and inhibits cell migration.
AUTHOR: Tan E; Gurjar M V; Sharma R V; Bhalla R C
CORPORATE SOURCE: Department of Anatomy and Cell Biology, University of Iowa College of Medicine, Iowa City 52242, USA.
CONTRACT NUMBER: HL-14388 (NHLBI)
HL-51735 (NHLBI)
SOURCE: CARDIOVASCULAR RESEARCH, (1999 Aug 15) 43 (3) 788-97.
Journal code: 0077427. ISSN: 0008-6363.
PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY DATE: Entered STN: 20000327
Last Updated on STN: 20000327
Entered Medline: 20000310

AB OBJECTIVES: It has been suggested that estrogen may improve endothelial cell function to delay the onset of atherosclerosis in pre-menopausal females, though its mechanism of action is not fully understood. We examined the hypothesis that human estrogen receptor-alpha (ER alpha) gene transfection improves the endothelial cell function. METHODS: A replication deficient adenoviral vector was used to transfect the ER alpha gene into bovine aortic endothelial cells (BAEC) and a GFP gene containing vector was used as **control**. Expression of the eNOS gene was **determined** by Northern blot analysis and **enzyme activity assay**; cell migration was assayed using a Transwell apparatus; and tyrosine phosphorylation of FAK was estimated by Western blot analysis. RESULTS: ER alpha gene transfection of endothelial cells produced a 2-3-fold increase in eNOS mRNA and protein levels as well as a significant increase ($P < 0.05$) in NOS activity as **measured** by citrulline assay and nitrite accumulation in the media in response to bradykinin stimulation. Treatment of cells with estrogen blocking agent ICI 182780 inhibited eNOS induction in response to ER alpha transfection. ER alpha gene transfection significantly inhibited ($P < 0.05$) bFGF-induced chemotactic migration of endothelial cells but increased cell attachment to fibronectin, laminin, and type I and IV collagens. ER alpha gene transfer also inhibited bFGF-stimulated tyrosine phosphorylation of FAK. CONCLUSION: Our results suggest that the atheroprotective effects of estrogen may in part be mediated by ER alpha-induced upregulation of eNOS gene expression and maintenance of endothelial cell function and integrity.

L26 ANSWER 29 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:490343 BIOSIS
DOCUMENT NUMBER: PREV199900490343
TITLE: Structural basis for selective inhibition of Src family **kinases** by PP1.
AUTHOR(S): Liu, Yi; Bishop, Anthony; Witucki, Laurie; Kraybill, Brian; Shimizu, Eiji; Tsien, Joe; Ubersax, Jeff; Blethrow, Justin; Morgan, David O.; Shokat, Kevan M. [Reprint author]
CORPORATE SOURCE: Department of Cellular and Molecular Pharmacology, UC San Francisco, San Francisco, CA, 94143-0450, USA
SOURCE: Chemistry and Biology (London), (Sept., 1999) Vol. 6, No. 9, pp. 671-678. print.
ISSN: 1074-5521.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Nov 1999
Last Updated on STN: 16 Nov 1999

AB Background: Small-molecule inhibitors that can target individual **kinases** are powerful tools for use in signal transduction research. It is difficult to find such compounds because of the enormous number of protein **kinases** and the highly conserved nature of their catalytic domains. Recently, a novel, potent, Src family selective tyrosine **kinase** inhibitor was reported (PP1). Here, we study the structural basis for this inhibitor's specificity for Src family **kinases**. Results: A single residue corresponding to Ile338 (v-Src numbering; Thr338 in c-Src) in Src family tyrosine **kinases** largely **controls** PP1's ability to inhibit protein

kinases. Mutation of Ile338 to a larger residue such as **methionine** or phenylalanine in v-Src makes this inhibitor less potent. Conversely, mutation of Ile338 to alanine or glycine increases PP1's potency. PP1 can inhibit Ser/Thr **kinases** if the residue corresponding to Ile338 in v-Src is mutated to glycine. We have accurately predicted several non-Src family **kinases** that are moderately (IC50 apprx 1 muM) inhibited by PP1, including c-Abl and the MAP **kinase p38**. Conclusions: Our mutagenesis studies of the ATP-**binding** site in both tyrosine **kinases** and Ser/Thr **kinases** explain why PP1 is a specific inhibitor of Src family tyrosine **kinases**. Determination of the structural basis of inhibitor specificity will aid in the design of more potent and more selective protein **kinase** inhibitors. The ability to desentisize a particular **kinase** to PP1 inhibition of residue 338 or conversely to sensitize a **kinase** to PP1 inhibition by mutation should provide a useful basis for chemical genetic studies of **kinase** signal transduction.

L26 ANSWER 30 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:362534 BIOSIS
DOCUMENT NUMBER: PREV199900362534
TITLE: Inhibition by tectorigenin and tectoridin of prostaglandin E2 production and **cyclooxygenase**-2 induction in rat peritoneal macrophages.
AUTHOR(S): Kim, Yong Pil; Yamada, Masateru; Lim, Soon Sung; Lee, Sang Hyun; Ryu, Nama; Shin, Kuk Hyun; Ohuchi, Kazuo [Reprint author]
CORPORATE SOURCE: Department of Pathophysiological Biochemistry, Graduate School of Pharmaceutical Sciences, Tohoku University, Aoba Aramaki, Aoba-ku, Sendai, Miyagi, 980-8578, Japan
SOURCE: Biochemica et Biophysica Acta, (June 10, 1999) Vol. 1438, No. 3, pp. 399-407. print.
ISSN: 0008-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 2 Sep 1999
Last Updated on STN: 2 Sep 1999

AB Tectorigenin and tectoridin, isolated from the rhizomes of Korean Belamcanda chinensis (Iridaceae) which are used as Chinese traditional medicine for the treatment of inflammation, suppressed prostaglandin E2 production by rat peritoneal macrophages stimulated by the protein **kinase** C activator, 12-O-tetradecanoylphorbol 13-acetate (TPA), or the endomembrane Ca2+-ATPase inhibitor, thapsigargin. Tectorigenin inhibited prostaglandin E2 production more potently than tectoridin. Neither compound inhibited the release of radioactivity from (3H)arachidonic acid-labeled macrophages stimulated by TPA or thapsigargin. In addition, activities of isolated **cyclooxygenase** (COX)-1 and COX-2 were not inhibited by the two compounds. Western blot analysis revealed that the induction of COX-2 by TPA or thapsigargin was inhibited by the two compounds in parallel with the inhibition of prostaglandin E2 production. These findings suggest that one of the mechanisms of the anti-inflammatory activities of the rhizomes of Belamcanda chinensis is the inhibition of prostaglandin E2 production by tectorigenin and tectoridin due to the inhibition of the induction of COX-2 in the inflammatory cells.

L26 ANSWER 31 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:288413 BIOSIS
DOCUMENT NUMBER: PREV199900288413
TITLE: Alteration of human leukotriene A4 hydrolase activity after

site-directed mutagenesis: Serine-415 is a regulatory residue.
AUTHOR(S): Rybina, Irina V.; Feinmark, Steven J. [Reprint author]
CORPORATE SOURCE: Department of Pharmacology, Columbia University, 630 West 168th Street, New York, NY, 10032, USA
SOURCE: Biochimica et Biophysica Acta, (May 18, 1999) Vol. 1438, No. 2, pp. 199-203. print.
CODEN: BBACAQ. ISSN: 0006-3002.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Aug 1999
Last Updated on STN: 5 Aug 1999

AB Leukotriene A4 hydrolase (LTA-H) is a bifunctional protein that has **aminopeptidase** activity, but also contains an epoxide hydrolase activity that converts leukotriene (LT)A4 to LTB4. The lipid metabolic activity of this **enzyme** plays a central role in the **control** of polymorphonuclear leukocyte function and in the development of inflammation. LTA-H is widely spread in many mammalian tissues, although it appears to be inactive in many cases. Regulation of this **enzyme**'s activity by phosphorylation of a serine at residue 415 has recently been described. Since the activation of LTA-H in the presence of activated PMNL would likely lead to a substantial increase in the production of inflammatory lipids, regulation of LTA-H presents a novel potential target for anti-inflammatory therapy. We have now made a series of site-directed mutants at this site to test the importance of this residue to the activity of LTA-H. Replacement of the critical serine with threonine or glutamine has little effect on either the epoxide hydrolase or **aminopeptidase** activities. However, replacing serine with a negatively charged amino acid (either aspartate or glutamate), intended to mimic phosphorylation at that site, causes significant reduction in epoxide hydrolase activity (50-70%). These mutations have little effect on the **aminopeptidase** activity of the LTA-H, suggesting that the mutation models the regulatory event and is not simply due to improper folding of the protein.

L26 ANSWER 32 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:88146 BIOSIS
DOCUMENT NUMBER: PREV200000088146
TITLE: Radiochemical high-performance **liquid** chromatographic assay for the determination of catechol O-methyltransferase activity towards various substrates.
AUTHOR(S): Lautala, Pia [Reprint author]; Ulmanen, Ismo; Taskinen, Jyrki
CORPORATE SOURCE: Department of Pharmacy, Division of Pharmaceutical Chemistry, University of Helsinki, FIN-00014, Helsinki, Finland
SOURCE: Journal of Chromatography B, (Dec. 24, 1999) Vol. 736, No. 1-2, pp. 143-151. print.
CODEN: JCBADL. ISSN: 0378-4347.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 10 Mar 2000
Last Updated on STN: 3 Jan 2002

AB A new chromatographic catechol O-methyltransferase (COMT) assay based on S-adenosyl-L-(methyl-14C)**methionine** and on-line radioactivity **detection** was developed. With minor modifications in the mobile phase composition the methylation velocities for 30 structurally diverse compounds including simple catechols, neurotransmitters, catecholestrogens and catecholic drugs could be **measured** using human and rat recombinant soluble COMT. The **enzymes** showed very similar

substrate selectivities. The radiochemical method was validated using 3,4-dihydroxybenzoic acid as a model substrate and it was shown that accurate and reproducible methylation velocity values could be achieved for both of the catecholic hydroxyls. The method proved to be suited for determining the **enzyme** kinetic parameters and can probably be further used for gathering **enzyme** kinetic data on differentially substituted catechols in order to construct proper structure-activity relationships for COMT.

L26 ANSWER 33 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:203522 BIOSIS
DOCUMENT NUMBER: PREV199900203522
TITLE: The roles of tryptophan and histidine residues in the catalytic activities of beta-cyclodextrin glucanotransferase from Bacillus firmus var. alkalophilus.
AUTHOR(S): Shin, Hyun-Dong; Kim, Chan; Lee, Yong-Hyun [Reprint author]
CORPORATE SOURCE: Department of Genetic Engineering, College of Natural Sciences, Kyungpook National University, Taegu, 702-701, South Korea
SOURCE: Journal of Microbiology and Biotechnology, (Feb., 1999) Vol. 9, No. 1, pp. 62-69. print.
ISSN: 1017-7825.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 26 May 1999
Last Updated on STN: 26 May 1999

AB In order to investigate the critical amino acid residues involved in the catalytic activities of beta-cyclodextrin glucanotransferase (beta-**CGTase**) excreted by Bacillus firmus var. alkalophilus, the amino acid residues in beta-**CGTase** were modified by various site-specific amino acid modifying reagents. The cyclizing and amylolytic activities of beta-**CGTase** were all seriously reduced after treatment with Woodward's reagent K (WRK) modifying aspartic/glutamic acid, N-bromosuccinimde (NBS) modifying tryptophan, and diethylpyrocarbonate (DEPC) modifying histidine residues. The roles of tryptophan and histidine residues in beta-**CGTase** were further investigated by **measuring** the protection effect of various substrates during chemical modification, comparing protein mobility in native and affinity polyacrylamide gel electrophoresis containing soluble starch, and comparing the Km and Vmax values of native and modified **enzymes**. Tryptophan residues were identified as affecting substrate-**binding** ability rather than influencing catalytic activities. On the other hand, histidine residues influenced catalytic ability rather than substrate-**binding** ability, plus histidine modification had an effect on shifting the optimum pH and pH stability.

L26 ANSWER 34 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:317452 BIOSIS
DOCUMENT NUMBER: PREV199900317452
TITLE: Intracellular **measurement** of prostaglandin E2: Effect of anti-inflammatory drugs on **cyclooxygenase** activity and prostanoid expression.
AUTHOR(S): Horton, Jeffrey K. [Reprint author]; Williams, Angela S.; Smith-Phillips, Zoe; Martin, Rhian C.; O'Beirne, Gerard
CORPORATE SOURCE: Amersham Pharmacia Biotech UK Ltd., Forest Farm, Whitchurch, Cardiff, CF4 7YT, UK
SOURCE: Analytical Biochemistry, (June 15, 1999) Vol. 271, No. 1, pp. 18-28. print.
CODEN: ANBCA2. ISSN: 0003-2697.
DOCUMENT TYPE: Article

LANGUAGE: English
ENTRY DATE: Entered STN: 17 Aug 1999
Last Updated on STN: 17 Aug 1999

AB **Cyclooxygenase** (COX) converts arachidonic acid to prostaglandin (PG) H2, which is further metabolized to various prostaglandins, prostacyclin and thromboxane A2. COX exists in at least two different isoforms. COX-1 is constitutively expressed, whereas COX-2 is induced by proinflammatory stimuli. Prostaglandin E2 is a major metabolite of COX activation. In order to compare the activity of target ligands and COX inhibitors on PGE2 synthesis and release, the responsiveness of several cell lines to the calcium ionophore A23187, bacterial lipopolysaccharide (LPS), nonsteroidal anti-inflammatory drugs (NSAIDs), and the glucocorticoid, dexamethasone, were investigated. For intracellular **measurements**, the culture supernatant was aspirated, and the cells were thoroughly washed and lysed with dodecyltrimethylammonium bromide. Intracellular and secreted PGE2 were **measured** with an **enzyme** immunoassay. A23187 and LPS increased intracellular PGE2 in a dose-dependent manner. Kinetic experimentswith A23187-stimulated mouse 3T3 fibroblast cells revealed a distinct biphasic response in COX activity. In the presence of NSAIDs or dexamethasone, there was a dose-dependent inhibition in intracellular PGE2 with A23187-stimulated 3T3 cells. Inhibitory studies demonstrated an apparent increased sensitivity of COX activity to the action of inhibitors when **measuring** intracellular PGE2 compared with using cell culture supernatants. Indeed, intracellular PGE2 levels were comprehensively reduced in the presence of low concentrations of inhibitor. The utilization of cell culture lysates and, in particular, **measurement** of intracellular PGE2 should prove useful for identifying new COX inhibitors.

L26 ANSWER 35 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:1549 BIOSIS
DOCUMENT NUMBER: PREV199900001549
TITLE: Repression of **cyclooxygenase**-2 and prostaglandin E2 release by dexamethasone occurs by transcriptional and post-transcriptional mechanisms involving loss of polyadenylated mRNA.
AUTHOR(S): Newton, Robert [Reprint author]; Seybold, Joachim; Kuitert, Lieske M. E.; Bergmann, Martin; Barnes, Peter J.
CORPORATE SOURCE: Dep. Thoracic Med., National Heart Lung Inst., Imperial Coll. Sch. Med., Dovehouse St., London SW3 6LY, UK
SOURCE: Journal of Biological Chemistry, (Nov. 27, 1998) Vol. 273, No. 48, pp. 32312-32321. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 11 Jan 1999
Last Updated on STN: 11 Jan 1999

AB The two **cyclooxygenase** (COX) isoforms convert arachidonic acid to precursor prostaglandins (PGs). Upregulation of COX-2 is responsible for increased PG production in inflammation and is antagonized by corticosteriods such as dexamethasone. In human pulmonary A549 cells, interleukin-1beta (IL-1beta) increases prostaglandin E2 (PGE2) synthesis via dexamethasone-sensitive induction of COX-2. Nuclear run-off assays showed that COX-2 transcription rate was repressed 25-40% by dexamethasone, while PGE, release, COX activity, and COX-2 protein were totally repressed. At the mRNA level, complete repression of COX-2 was only observed at later (6 h) **time** points. Preinduced COX-2 mRNA was also potently repressed by dexamethasone, yet suppression of transcription by actinomycin D showed little effect. This dexamethasone-dependent repression involved a reduced COX-2 mRNA

half-life, was blocked by actinomycin D or cycloheximide, and was antagonized by the steroid antagonist RU38486. Repression of IL-1beta-induced PGE2 release, COX activity, and COX-2 protein by actinomycin D was only effective within the first hour following IL-1beta treatment, while dexamethasone was effective when added up to 10 h later, suggesting a functional role for post-transcriptional mechanisms of repression. Following dexamethasone treatment, shortening of the average length of COX-2 mRNA poly(A) tails was observed. Finally, ligation of the COX-2 3'-UTR to a heterologous reporter failed to confer dexamethasone sensitivity. In conclusion, these data indicate a major role for post-transcriptional mechanisms in the dexamethasone-dependent repression of COX-2 that require de novo glucocorticoid receptor-dependent transcription and translation. This mechanism involves shortening of the COX-2 poly(A) tail and requires determinants other than just the 3'-UTR for specificity.

L26 ANSWER 36 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:494772 BIOSIS
DOCUMENT NUMBER: PREV199800494772
TITLE: Calmodulin-dependent regulation of inducible and neuronal **nitric-oxide synthase**.
AUTHOR(S): Lee, Shiow-Ju; Stull, James T. [Reprint author]
CORPORATE SOURCE: Dep. Physiol., Univ. Texas Southwestern Med. Cent. Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235-9040, USA
SOURCE: Journal of Biological Chemistry, (Oct. 16, 1998) Vol. 273, No. 42, pp. 27430-27437. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 18 Nov 1998
Last Updated on STN: 18 Nov 1998

AB Neuronal and endothelial **nitric-oxide synthases** depend upon Ca2+/calmodulin for activation, whereas the activity of the inducible **nitric-oxide synthase** is Ca2+independent, presumably due to tightly **bound** calmodulin. To study these different mechanisms, a series of chimeras derived from neuronal and inducible **nitric-oxide synthases** were analyzed. Chimeras containing only the oxygenase domain, calmodulin-**binding** region, or reductase domain of inducible **nitric-oxide synthase** did not confer significant Ca2+-independent activity. However, each chimera was more sensitive to Ca2+ than the neuronal isoform. The calmodulin-**binding** region of inducible **nitric-oxide synthase** with either its oxygenase or reductase domains resulted in significant, but not total, Ca2+-independent activity. Co-immunoprecipitation experiments showed no calmodulin associated with the former chimera in the absence of Ca2+. Trifluoperazine also inhibited this chimera in the absence of Ca2+. The combined interactions of calmodulin **bound** to inducible **nitric-oxide synthase** calmodulin-**binding** region with the oxygenase domain may be weaker than with the reductase domain. Thus, Ca"-independent activity of inducible **nitric-oxide synthase** appears to result from the concerted interactions of calmodulin with both the oxygenase and reductase domains in addition to the canonical calmodulin-**binding** region. The neuronal isoform is not regulated by a unique autoinhibitory element in its reductase domain.

L26 ANSWER 37 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:319424 BIOSIS

DOCUMENT NUMBER: PREV199800319424
TITLE: Autocrine regulation of inducible **nitric-oxide synthase** in macrophages by atrial natriuretic peptide.
AUTHOR(S): Kiemer, Alexandra K.; Vollmar, Angelika M. [Reprint author]
CORPORATE SOURCE: Inst. Pharmacol. Toxicol. Pharm., Univ. Munich, Koeniginstr. 16, 80539 Munich, Germany
SOURCE: Journal of Biological Chemistry, (May 29, 1998) Vol. 273, No. 22, pp. 13444-13451. print.
CODEN: JBCHA3. ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Jul 1998
Last Updated on STN: 22 Jul 1998

AB Atrial natriuretic peptide (ANP), a cardiovascular hormone, has been shown to inhibit synthesis of nitric oxide in lipopolysaccharide (LPS)-activated mouse bone marrow-derived macrophages via activation of its guanylate cyclase-**coupled** receptor. The goal of the present study was to elucidate the potential sites of inducible **nitric-oxide synthase** (iNOS) regulation affected by ANP and revealed the following. 1) ANP and dibutyryl-cGMP did not inhibit catalytic iNOS activity **measured** by the conversion rate of L-(3H)arginine to L-(3H)citrulline in homogenates of LPS-treated. cells. 2) Pretreatment of cells with ANP dose-dependently reduced the LPS-induced L-(3H)citrulline production that has been shown to be due to reduced iNOS protein levels **detected** by Western blot. 3) ANP does not alter the ratio of catalytically active iNOS dimer versus inactive iNOS monomer considered to be a major post-translational regulatory mechanism for the **enzyme** . 4) Macrophages exposed to ANP display decreased LPS-induced iNOS mRNA levels. 5) Importantly, two basic mechanisms seem to be responsible for this observation, i.e. ANP specifically induced acceleration of iNOS mRNA decay and ANP reduced **binding** activity of NF-kappaB, the transcription factor predominantly responsible for LPS-induced iNOS expression in murine macrophages. Moreover, 6) ANP acts via an autocrine mechanism since recently ANP was shown to be secreted by LPS-activated macrophages, and we demonstrated here that LPS-induced NO synthesis was increased after blocking the **binding** of endogenous ANP by a receptor antagonist. These observations suggest ANP as a new autocrine macrophage factor regulating NO synthesis both transcriptionally and post-transcriptionally. ANP may help to balance NO production of activated macrophages and thus may allow successful immune response without adverse effects on host cells.

L26 ANSWER 38 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1998:485932 BIOSIS
DOCUMENT NUMBER: PREV199800485932
TITLE: Characterization of beta-galactosidase from a Bacillus sp. with high catalytic efficiency for transgalactosylation.
AUTHOR(S): In, Man-Jin; Jung, Jin [Reprint author]
CORPORATE SOURCE: Dep. Agric. Chem., Seoul Natl. Univ., Suwon 441-744, South Korea
SOURCE: Journal of Microbiology and Biotechnology, (Aug., 1998) Vol. 8, No. 4, pp. 318-324. print.
ISSN: 1017-7825.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 5 Nov 1998
Last Updated on STN: 5 Nov 1998

AB A beta-galactosidase with high transgalactosylic activity was purified from a Bacillus species, registered as KFCC10855. The **enzyme**

preparation showed a single protein band corresponding to a molecular mass of 150 kDa on SDS-PAGE and gave a single peak with the estimated molecular mass of 250 kDa on Sephacryl S-300 gel filtration, suggesting that the **enzyme** is a homodimeric protein. The amino acid and sugar analyses revealed that the **enzyme** is a glycoprotein, containing 19.2 weight percent of sugar moieties, and is much more abundant in hydrophilic amino acid residues than in hydrophobic residues, the mole ratio being about 2:1. The pI and optimum pH were **determined** to be 5.0 and 6.0, respectively. Having a temperature optimum at 70degreeC for the hydrolysis of lactose, the **enzyme** showed good thermal stability. The activity of the **enzyme** preparation was markedly increased by the presence of exogenous Mg (II) and was decreased by the addition of EDTA. Among the metal ions examined, the most severely inhibitory effect was seen with Ag (I) and Hg (II). Further, results of protein modification by various chemical reagents implied that 1 cysteine, 1 histidine, and 2 **methionine** residues occur in certain critical sites of the **enzyme**, most likely including the active site. **Enzyme** kinetic parameters, **measured** for both hydrolysis and transgalactosylation of lactose, indicated that the **enzyme** has an excellent catalytic efficiency for formation of the transgalactosylic products in reaction mixtures containing high concentrations of the substrate.

L26 ANSWER 39 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:27993 BIOSIS
DOCUMENT NUMBER: PREV199900027993
TITLE: **Detection** of native and recombinant Bacillus macerans cyclodextrin glycosyltransferase using microtiter ELISA techniques.
AUTHOR(S): Han, Nam Soo; Tao, Bernard Y. [Reprint author]
CORPORATE SOURCE: Dep. Agric. and Biol. Eng., Purdue Univ., 1146 Agricultural Eng. Build., W. Lafayette, IN 47907, USA
SOURCE: Enzyme and Microbial Technology, (Jan.-Feb. 1, 1998) Vol. 24, No. 1-2, pp. 35-40. print.
CODEN: EMTED2. ISSN: 0141-0229.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Feb 1999
Last Updated on STN: 3 Feb 1999

AB Direct, sandwich, and competitive ELISA methods were developed to quantify Bacillus macerans cyclodextrin glycosyltransferase (**CGTase**) and its thioredoxin fusion molecule using rabbit polyclonal antibodies. The direct ELISA method was used to demonstrate equivalent molar response of both native **CGTase** and the thioredoxin-**CGTase** fusion protein to antibody **binding**. Sandwich ELISA showed the most sensitive **detection** range (0.2 apprx 50 mug ml-1) against **CGTase**. We improved a competitive ELISA method by coating the microtiter plate with denatured **CGTase** in 6 M guanidine-HCl **solution**, resulting in enhanced, rapid response. This competitive ELISA method was specific, precise, and rapid when the method was used to quantify **CGTase** and monitor production of **CGTase** and its thioredoxin fusion protein in the recombinant E. coli. These methods are specifically useful when **detecting** and quantifying recombinant **CGTase** proteins in which mutations may have reduced or eliminated enzymatic activity.

L26 ANSWER 40 OF 51 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2000394827 MEDLINE
DOCUMENT NUMBER: 20376465 PubMed ID: 10921051
TITLE: Effect of up-regulation of S-AdoMet synthetase on

taxol-induced apoptosis in human breast cancer cells.
AUTHOR: Chen L; Zheng S; Fan W
CORPORATE SOURCE: Cancer Institute, Zhejiang Medical University, Hangzhou.
SOURCE: CHUNG-HUA CHUNG LIU TSA CHIH [CHINESE JOURNAL OF ONCOLOGY], (1998 Jan) 20 (1) 28-30.
Journal code: 7910681. ISSN: 0253-3766.
PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY DATE: Entered STN: 20000824
Last Updated on STN: 20000824
Entered Medline: 20000811

AB OBJECTIVE: To investigate the gene regulation of taxol-induced apoptosis. METHODS: Northern blot hybridization, **enzyme activity assay** of S-AdoMet synthetase and flow cytometry were performed in the investigation of expression in the mRNA level and biological action of S-AdoMet synthetase in taxol-induced apoptosis in human breast cancer cell line (BCap 37). RESULTS: Up-regulation of S-AdoMet synthetase expression was resulted by taxol treatment and the expression peaked at 48 hours. Moreover, the up-regulation of S-AdoMet synthetase was associated with cytotoxicity of antimicrotubule agents including taxol and colchicine. Inhibition rate of S-AdoMet synthetase activity by 1% DMSO was 34% in taxol-treated cells and 14% in taxol-untreated cells compared to **control** groups, respectively. Posttreatment with 1% DMSO following pretreatment with individual antitumor agent for 3 hr promoted apoptotic cell death of taxol-, colchicine-, and adriamycin-treated BCap37 cells. CONCLUSION: The induction of apoptosis enhanced by post-treatment with DMSO in taxol-treated cells is probably linked to its inhibition on **enzyme** activity of S-AdoMet synthetase, suggesting that the increased expression of S-AdoMet synthetase possibly plays an important role in protecting cells from DNA fragmentation in taxol-induced apoptosis.

L26 ANSWER 41 OF 51 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 97322030 MEDLINE
DOCUMENT NUMBER: 97322030 PubMed ID: 9178684
TITLE: Overexpressed **nitric oxide synthase** in portal-hypertensive stomach of rat: a key to increased susceptibility to damage?.
AUTHOR: Ohta M; Tanoue K; Tarnawski A S; Pai R; Itani R M; Sander F C; Sugimachi K; Sarfeh I J
CORPORATE SOURCE: Department of Surgery, Department of Veterans Affairs Medical Center, Long Beach, California 90822, USA.
SOURCE: GASTROENTEROLOGY, (1997 Jun) 112 (6) 1920-30.
Journal code: 0374630. ISSN: 0016-5085.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970805
Last Updated on STN: 19970805
Entered Medline: 19970721

AB BACKGROUND & AIMS: Portal hypertension predisposes gastric mucosa to increased injury. The aim of this study was to **determine** whether overexpression of constitutive **nitric oxide synthase** (cNOS) is responsible for increased susceptibility of portal-hypertensive (PHT) gastric mucosa to damage. METHODS: In gastric

specimens from PHT and sham-operated rats, cNOS messenger RNA expression was **determined** by Northern blotting and cNOS protein expression by Western blotting, immunohistochemistry, and **enzyme activity assay**. Extent of ethanol-induced gastric mucosal necrosis, mucosal blood flow, and gastric NOS activity in PHT and sham-operated rats was **determined** after administration of N(omega)-nitro-L-arginine methyl ester (L-NAME) or saline. RESULTS: cNOS messenger RNA level, cNOS **enzyme** activity, and fluorescence signals for cNOS were increased significantly in PHT rats compared with **controls**. Inhibition of overexpressed cNOS by L-NAME (5 mg/kg) significantly reduced ethanol-induced mucosal necrosis and normalized blood flow in PHT gastric mucosa, whereas this dose of L-NAME significantly increased mucosal necrosis in sham-operated rats. CONCLUSIONS: Portal hypertension activates the cNOS gene with overexpression of cNOS protein in endothelia of gastric mucosal vessels. Excessive NO production by overexpressed cNOS may play an important role in the increased susceptibility of PHT gastric mucosa to damage.

L26 ANSWER 42 OF 51 MEDLINE on STN
ACCESSION NUMBER: 97279852 MEDLINE
DOCUMENT NUMBER: 97279852 PubMed ID: 9134226
TITLE: Nitric oxide modulation of transcellular biosynthesis of cys-leukotrienes in rabbit leukocyte-perfused heart.
AUTHOR: Buccellati C; Rossoni G; Bonazzi A; Berti F; Maclouf J; Folco G; Sala A
CORPORATE SOURCE: Center for Cardiopulmonary Pharmacology, Institute of Pharmacological Sciences, Milan, Italy.
SOURCE: BRITISH JOURNAL OF PHARMACOLOGY, (1997 Mar) 120 (6) 1128-34.
Journal code: 7502536. ISSN: 0007-1188.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199708
ENTRY DATE: Entered STN: 19970908
Last Updated on STN: 19970908
Entered Medline: 19970827

AB 1. We have studied the role of nitric oxide (NO) in the regulation of the transcellular biosynthesis of sulphidopeptide leukotrienes (cys-LT) generated upon neutrophil-vascular wall interactions and their functional consequences, in the spontaneously beating, cell-perfused, heart of the rabbit. 2. Hearts were perfused under recirculating conditions (50 ml) with 5 x 10(6) purified human neutrophils (PMNL), and challenged with 0.5 microM A-23187 for 30 min. Coronary perfusion pressure (CPP) and left-ventricular end-diastolic pressure (LVEDP) were monitored. Cys-LT formation was **measured** by reversed phase high performance liquid chromatography (h.p.l.c.) and u.v. spectral analysis. Myeloperoxidase (MPO) **enzyme activity, assayed** in aliquots of the recirculating buffer, was used as a marker of PMNL, adhesion to the coronary endothelium. 3. Basal CPP and LVEDP values averaged 45 +/- 1.4 mmHg and 5 +/- 0.1 mmHg, respectively; A-23187 triggered an increase in CPP (134 +/- 9 mmHg, at 30 min) which was significantly attenuated by pretreatment with L-arginine, 100 microM (90 +/- 3 mmHg, at 30 min). Pretreatment with NG-monomethyl-L-arginine, 10 microM (L-NMMA), induced a marked increase in CPP (290 +/- 40 mmHg, at 20 min) and in LVEDP (47 +/- 16 mmHg), so pronounced that it caused cardiac arrest in systole in 5 out of 6 hearts and these were prevented by L-arginine, 100 microM, (CPP 115 +/- 10 mmHg, LVEDP 6 +/- 1.1 mmHg, at 30 min). 4. The increase in CPP was accompanied by the release of cys-LT in the circulating buffer, which was

reduced significantly by L-arginine. Pretreatment with L-NMMA, caused a marked rise in cys-LT concentrations which was prevented by L-arginine. 5. Neither L-arginine nor L-NMMA affected directly the A-23187-induced arachidonic acid (AA) metabolism in isolated PMNL alone. 6. Pretreatment with L-NMMA caused a prompt drop in myeloperoxidase (MPO), activity, suggesting rapid adhesion of PMNL to the coronary wall; this effect was significantly blunted by L-arginine. 7. This study suggests that NO provides cardioprotection in an organ model of transcellular metabolism of cys-LT by preventing PMNL adhesion to the coronary intima.

L26 ANSWER 43 OF 51 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 97098190 MEDLINE
DOCUMENT NUMBER: 97098190 PubMed ID: 8942731
TITLE: Helicobacter pylori stimulates inducible **nitric oxide synthase** expression and activity in a murine macrophage cell line.
AUTHOR: Wilson K T; Ramanujam K S; Mobley H L; Musselman R F; James S P; Meltzer S J
CORPORATE SOURCE: Department of Medicine, University of Maryland School of Medicine, Baltimore, USA.. kwilson@umabnet.ab.umd.edu
CONTRACT NUMBER: DK47717 (NIDDK)
ES07120 (NIEHS)
R01-CA67497 (NCI)
+
SOURCE: GASTROENTEROLOGY, (1996 Dec) 111 (6) 1524-33.
Journal code: 0374630. ISSN: 0016-5085.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970128
Last Updated on STN: 19970128
Entered Medline: 19970102

AB BACKGROUND & AIMS: Helicobacter pylori uniquely colonizes the human stomach and produces gastric mucosal inflammation. High-output nitric oxide production by inducible **nitric oxide synthase** (iNOS) is associated with immune activation and tissue injury. Because mononuclear cells comprise a major part of the cellular inflammatory response to H. pylori infection, the ability of H. pylori to induce iNOS in macrophages was assessed. METHODS: H. pylori preparations were added to RAW 264.7 murine macrophages, and iNOS expression was assessed by Northern blot analysis, **enzyme activity assay**, and NO2- release. RESULTS: Both whole H. pylori and French press lysates induced concentration-dependent NO2- production, with peak levels 20-fold above **control**. These findings were paralleled by marked increases in iNOS messenger RNA and **enzyme** activity levels. iNOS expression was synergistically increased with interferon gamma, indicating that the H. pylori effect can be amplified by other macrophage-activating factors. Studies of lipopolysaccharide (LPS) content and polymyxin B inhibition of LPS suggested that the H. pylori effect was attributable to both LPS-dependent and -independent mechanisms. CONCLUSIONS: iNOS expression in macrophages is activated by highly stable H. pylori products and may play an important role in the pathogenesis of H. pylori-associated gastric mucosal disease.

L26 ANSWER 44 OF 51 JAPIO (C) 2004 JPO on STN
ACCESSION NUMBER: 1994-220002 JAPIO
TITLE: HYDROXYPHENYLGLYCINE DERIVATIVE AND **ENZYME ACTIVITY ASSAY** USING THE SAME

INVENTOR: UEMATSU HITOSHI; CHIBA HIROYUKI; NAKAJIMA TAKASHI; SHIBAMOTO NORIO; YOSHIOKA TAKEO; OKAMURA KAZUHIKO; OKAMOTO ROKURO; SHIN TAKASHI; MURAO SAWAO
PATENT ASSIGNEE(S): MERCIAN CORP
PATENT INFORMATION:

PATENT NO	KIND	DATE	ERA	MAIN IPC
JP 06220002	A	19940809	Heisei	C07C229-36

APPLICATION INFORMATION
STN FORMAT: JP 1993-28594 19930125
ORIGINAL: JP05028594 Heisei
PRIORITY APPLN. INFO.: JP 1993-28594 19930125
SOURCE: PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 1994

AN 1994-220002 JAPIO
AB PURPOSE: To provide the subject new compound useful as a new substrate to be used for the assays of **enzyme** activities such as angiotensinase activity or **aminopeptidase** activity.
CONSTITUTION: The objective compound of formula I [R<SP>1</SP> is H, t-butyloxycarbonyl, benzoyl or L-leucyl of formula II (R<SP>3</SP> is H, t-butyloxycarbonyl or benzyloxycarbonyl); R<SP>2</SP> is OH, benzyloxy or L-histidyl-L-leucyl of formula III (R<SP>4</SP> is H or alkyl)], e.g. N-benzoyl-L-2-(4-hydroxyphenyl)glycyl-L-histidyl-L- leucine. Angiotensinase is made to act on the above illustrated compound to form N-benzoyl-L-2-(4-hydroxyphenyl)glycine, which is then converted into 4-hydroxybenzaldehyde by an oxidase, and this product is quantified to **determine** angiotensinase activity. The activity of **aminopeptidase** can be **determined** using L-leucyl-L-2--(4-hydroxyphenyl)glycine as substrate.

L26 ANSWER 45 OF 51 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 94342755 MEDLINE
DOCUMENT NUMBER: 94342755 PubMed ID: 8064161
TITLE: Purification of endothelin-1-inactivating peptidase from the rat kidney.
AUTHOR: Janas J; Sitkiewicz D; Pulawska M F; Warnawin K; Janas R M
CORPORATE SOURCE: Department of Clinical Biochemistry, National Institute of Cardiology, Warsaw, Poland.
SOURCE: JOURNAL OF HYPERTENSION, (1994 Apr) 12 (4) 375-82.
Journal code: 8306882. ISSN: 0263-6352.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199409
ENTRY DATE: Entered STN: 19941005
Last Updated on STN: 20000303
Entered Medline: 19940920

AB OBJECTIVE: To identify and purify endothelin-1-inactivating peptidase from rat tissues. METHODS: Subcellular fractions of rat kidney, aorta, heart, lung, liver and blood cells were prepared by differential centrifugation. Kidney membrane-**bound** peptidase was solubilized with Triton X-100, chromatographed on the diethylaminoethyl-cellulose, ultrafiltered through a membrane of relative molecular mass 100,000 cutoff and subjected to electrophoresis on a non-denaturing polyacrylamide gel. The **enzyme activity assay** was performed at pH 5.5 using [125I]-endothelin-1 as the substrate. The trichloroacetic acid

precipitation test, an endothelin-1 immunoreactivity assay, reverse-phase high-performance **liquid** chromatography and a receptor-**binding** assay were applied for the **detection** of degradation products. RESULTS: High-activity endothelin-1-degrading peptidase coincided with the fraction from the kidney membranes of both Wistar-Kyoto and spontaneously hypertensive rats, but not with any other of the tissues that were studied. The membrane (0.5 microgram protein/assay) degraded [125I]-endothelin-1 (5-100 pmol/l) within a half-**time** of about 10 min at 37 degrees C. The **enzyme** was purified to an apparent homogeneity with non-denaturing gel electrophoresis, by which it was identified as a low-mobility (Rf 0.07) protein fraction of high relative molecular mass (> 250,000). The optimum pH was 5.5, with a little activity found outside the range 5.0-7.0. The activity of the peptidase was inhibited by 0.5 mmol/l 1,10 phenanthroline (half-maximal inhibitory concentration 0.03 mmol/l), and by 1 mmol/l EDTA, implicating a **metalloenzyme**. Bestatin, puromycin, phenylmethylsulphonyl fluoride and thiorphan were without effect. Unlabelled endothelin-1 inhibited the degradation of [125I]-endothelin-1 (half-maximal inhibitory concentration 100 nmol/l), whereas 100 mumol/l **methionine** enkephalin or angiotensin I did not. High-performance **liquid** chromatography analyses of the [125I]-endothelin-1 incubated with purified peptidase revealed a **time**-dependent accumulation of one major radioactive fraction that was soluble in trichloroacetic acid. This product (or products) was not further hydrolysed. It did not react with the endothelin antibodies or with the specific, myocardial membrane receptors. CONCLUSION: Our data suggest that the rat kidney contains an acidic metalloproteinase of high relative molecular mass that is able to hydrolyse endothelin-1 rapidly and efficiently in vitro. The **enzyme** may participate in the inactivation of circulating or tissue endothelins, or both.

L26 ANSWER 46 OF 51 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 93388873 MEDLINE
DOCUMENT NUMBER: 93388873 PubMed ID: 8376591
TITLE: Prevalence of autoantibodies to the 65- and 67-kD isoforms of glutamate decarboxylase in insulin-dependent diabetes mellitus.
AUTHOR: Seissler J; Amann J; Mauch L; Haubruck H; Wolfahrt S; Bieg S; Richter W; Holl R; Heinze E; Northemann W; +
CORPORATE SOURCE: Department of Internal Medicine, University of Ulm, Germany.
SOURCE: JOURNAL OF CLINICAL INVESTIGATION, (1993 Sep) 92 (3) 1394-9.
Journal code: 7802877. ISSN: 0021-9738.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199310
ENTRY DATE: Entered STN: 19931105
Last Updated on STN: 19931105
Entered Medline: 19931015

AB We investigated the presence of autoantibodies to baculovirus-expressed human recombinant 65- and 67-kD isoforms of glutamate decarboxylase (GAD65 and GAD67) in insulin-dependent diabetes mellitus (IDDM). In the immunoprecipitation test using [35S]**methionine**-labeled GADs antibodies to GAD65 were **detected** in 13/15 (87%) islet cell antibody (ICA)-positive and in 1/35 (2.9%) ICA-negative first-degree relatives of patients with IDDM, in 6/11 (54.5%) ICA-positive nondiabetic schoolchildren, and in 35/50 (70%) patients with newly diagnosed IDDM.

GAD67 antibodies were positive only in five (33%) of the ICA-positive relatives ($P < 0.05$) and in nine (18%) IDDM patients at onset ($P < 0.00001$). After onset of IDDM antibodies to GAD65 and GAD67 declined but were still positive in 25 and 9.4% of subjects with long-standing IDDM (> 10 yr). In all study groups antibodies to GAD67 were only **detected** in GAD65 antibody-positive sera. An immunotrapping **enzyme activity assay** for GAD65 antibodies was positive in 64/75 (85.3%) of sera that were GAD antibody positive in the immunoprecipitation test ($r = 0.870$, $P < 0.0001$). In two (2.7%) sera GAD65 antibodies that block GAD **enzyme** activity were found. Our data suggest that antibodies to GAD65 but not to GAD67 represent sensitive markers for preclinical and overt IDDM. The immunotrapping assay here described represents a valuable technique for specific and sensitive screening for GAD antibodies.

L26 ANSWER 47 OF 51 EMBASE
on STN

ACCESSION NUMBER: 94022056 EMBASE
DOCUMENT NUMBER: 1994022056
TITLE: Leishmania: Immunochemical comparison of the secretory (extracellular) acid phosphatases from various species.
AUTHOR: Doyle P.S.; Dwyer D.M.
CORPORATE SOURCE: Division of Infectious Diseases, University of California, San Francisco General Hospital, 1001 Potrero Avenue,San Francisco, CA 94110, United States
SOURCE: Experimental Parasitology, (1993) 77/4 (435-444). ISSN: 0014-4894 CODEN: EXPAAA
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Promastigotes of all Leishmania species, except most Leishmania major stocks, synthesize and secrete extracellular soluble acid phosphatases [SAcP(s)] which are capable of dephosphorylating a wide range of substrates and, consequently, of modifying the parasite environment. To assess their antigenic/structural relatedness, the SAcP(s) from various species were compared immunochemically using both a monospecific rabbit antiserum and several monoclonal antibodies made against the purified promastigote SAcP of a cloned line of Leishmania donovani donovani. Results obtained from antibody-bridged **enzyme activity assays** demonstrated that these reagents quantitatively immunoprecipitated the enzymatic activities of SAcP(s) from 18 different W.H.O. reference stocks of Leishmania, including two L. major strains. Those results showed, for the first **time**, that all SAcP(s) possessed some common cross-reactive antigenic epitopes. Immunoprecipitates obtained from [35S]**methionine**, metabolically labeled promastigote culture supernatants of the various species, were analyzed by SDS-PAGE/fluorography. These analyses showed that marked differences existed among the SAcP(s) both in their relative mobilities and the number of constituent bands resolved. Tunicamycin treatment resulted in a significant reduction in the apparent molecular weights of SAcP(s) from three different isolates, confirming the presence of N-linked carbohydrate side chains in these **enzymes**. In addition, the SAcP released by L. donovani intracellular amastigotes was also immunoprecipitated with the monospecific rabbit antisera from the culture supernatant of infected macrophages. Cumulatively, these data demonstrate that despite individual species variations, the SAcP(s) from all Leishmania tested have retained certain common antigenic/structural

epitopes and functional protein domains. Such conservation among SAcP(s) suggests that this **enzyme** must play an essential role in the survival of promastigotes and amastigotes of all Leishmania species.

L26 ANSWER 48 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 9
ACCESSION NUMBER: 1992:279316 BIOSIS
DOCUMENT NUMBER: PREV199294003966; BA94:3966
TITLE: **ENZYME** ACTIVITIES IN WASTE WATER AND ACTIVATED SLUDGE.
AUTHOR(S): NYBROE O [Reprint author]; JORGENSEN P E; HENZE M
CORPORATE SOURCE: WATER QUALITY INST, AGERN ALLE 11, DK-2970 HORSHOLM, DENMARK
SOURCE: Water Research, (1992) Vol. 26, No. 5, pp. 579-584.
CODEN: WATRAG. ISSN: 0043-1354.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 10 Jun 1992
Last Updated on STN: 10 Jun 1992

AB The purpose of the present study was to evaluate the potential of selected **enzyme activity assays** to **determine** microbial abundance and heterotrophic activity in waste water and activated sludge. In waste water, esterase and dehydrogenase activities were found to correlate with microbial abundance **measured** as colony forming units of heterotrophic bacteria. A panel of four **enzyme activity assays**, .alpha.-glucosidase, alanine-**aminopeptidase**, esterase and dehydrogenase were used to characterize activated sludge and anaerobic hydrolysis sludge from a pilot scale plant. The enzymatic activity profiles were distinctly different, suggesting that microbial populations were different, or had different physiological properties, in the two types of sludge. **Enzyme** activity profiles in activated sludge from four full-scale plants seemed to be highly influenced by the compositions of the inlet. Addition of hydrolysed starch was, for instance, reflected in a high .alpha.-glucosidase activity. However, no obvious correlations between specific process parameters and **enzyme** activities were found.

L26 ANSWER 49 OF 51 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 89034450 MEDLINE
DOCUMENT NUMBER: 89034450 PubMed ID: 3182940
TITLE: A role for glyceraldehyde-3-phosphate dehydrogenase in the development of thermotolerance in Xenopus laevis embryos.
AUTHOR: Nickells R W; Browder L W
CORPORATE SOURCE: Department of Biological Sciences, University of Calgary, Alberta, Canada.
SOURCE: JOURNAL OF CELL BIOLOGY, (1988 Nov) 107 (5) 1901-9.
Journal code: 0375356. ISSN: 0021-9525.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198812
ENTRY DATE: Entered STN: 19900308
Last Updated on STN: 19990129
Entered Medline: 19881201

AB During heat shock, Xenopus laevis embryos exhibit an increase in the rate of accumulation of lactate and a loss of ATP relative to non-heat-shocked **control** embryos. These results suggest that heat shock stimulates a shift in energy metabolism to anaerobic glycolysis while at the same

time causing an increase in the demand for ATP. We have evidence indicating that the embryo may meet such demands placed on it by increasing the levels of some glycolytic **enzymes**. In this report, we show that heat shock stimulates increases in the glycolytic **enzyme** glyceraldehyde-3-phosphate dehydrogenase [(EC 1.2.1.12] GAPDH). The specific activity of GAPDH shows a significant increase after heat shock, which correlates with the accumulation of GAPDH in heat-shocked embryos as **detected** by immunoblotting. Increases in GAPDH-specific activity are variable, however, and are inversely proportional to the levels of specific activity in **control** embryos; i.e., constitutive **enzyme** activity. We further analyzed the heat-enhanced accumulation of GAPDH by electrophoretically separating GAPDH isozymes on nondenaturing polyacrylamide gels. **Control** embryos exhibit a single isozyme of GAPDH, whereas heat-shocked embryos exhibit two isozymes of GAPDH. When these isozymes are labeled with [35S]**methionine**, separated by nondenaturing gel electrophoresis, and analyzed by fluorography, a heat-shock protein is found to comigrate with the isozyme unique to the heat-shocked sample. **Enzyme activity assays** at different temperatures suggest that this isozyme has optimum enzymatic activity only at heat-shock temperatures. We have correlated a 35-kD heat-shock protein (hsp35) with GAPDH using the following evidence: this hsp comigrates with GAPDH on one-dimensional SDS polyacrylamide gels; heat-enhanced increases in GAPDH specific activity correlate with hsp35 synthesis; and hsp35 and GAPDH have similar peptide maps. This relationship also provides a compelling explanation for the restriction of hsp35 synthesis to the vegetal hemisphere cells of heat-shocked early gastrulae reported previously (Nickells, R. W., and L. W. Browder. 1985. Dev. Biol. 112:391-395).

L26 ANSWER 50 OF 51 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1988:132614 BIOSIS
DOCUMENT NUMBER: PREV198885067441; BA85:67441
TITLE: A SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF GAMMA GLUTAMYL CYCLOTRANSFERASE WITH ALANINE DEHYDROGENASE IN THE PRESENCE OF ANTHGLUTIN.
AUTHOR(S): TAKAHASHI T [Reprint author]; KONDO T; OHNO H; MINATO S; OHSHIMA T; MIKUNI S; SODA K; TANIGUCHI N
CORPORATE SOURCE: DEP BIOCHEM, OSAKA UNIV MED SCH, NAKANOSHIMA, OSAKA 530, JAPAN
SOURCE: Biochemical Medicine and Metabolic Biology, (1987) Vol. 38, No. 3, pp. 311-316.
CODEN: BMMBES. ISSN: 0885-4505.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
ENTRY DATE: Entered STN: 12 Mar 1988
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AB .gamma.-Glutamyl cyclotransferase activity is assayed in tissues by a colorimetric method using .gamma.-glutamyl alanine as a substrate coupled with alanine dehydrogenase from Bacillus sphaericus, to measure the formation of NADH. In order to avoid interference by the reaction catalyzed by .gamma.-glutamyl transpeptide, anthglutin, a specific inhibitor of the transpeptide was included in the reaction mixture. The Km value of rat kidney .gamma.-glutamyl cyclotransferase with respect to .gamma.-glutamyl alanine appeared to be the same when determined by either the colorimetric or the radiometric method. This assay presents a reliable alternative to the use of **radiolabeled substrate** and is used for the assay of .gamma.-glutamyl cyclotransferase in a variety of physiological and experimental samples.

L26 ANSWER 51 OF 51 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
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ACCESSION NUMBER: 78375582 EMBASE
DOCUMENT NUMBER: 1978375582
TITLE: Studies of the cause and treatment of hyperammonemia in females with ornithine transcarbamylase deficiency.
AUTHOR: Glasgow A.M.; Kraegel J.H.; Schulman J.D.
CORPORATE SOURCE: Dept. Endocrinol. Metab., Child. Hosp., Nat. Med. Cent., Washington, D.C. 20010, United States
SOURCE: Pediatrics, (1978) 62/1 (30-37).
CODEN: PEDIAU
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 037 Drug Literature Index
007 Pediatrics and Pediatric Surgery
029 Clinical Biochemistry
022 Human Genetics
030 Pharmacology
LANGUAGE: English

AB Assay of ornithine transcarbamylase (OTC) activity in multiple small bits of liver (approximately 5 mg) that were obtained from a single surgical biopsy in a patient with OTC deficiency revealed a 10- to 40-fold variation in **enzyme** activity. Similar studies with **control** autopsy liver specimens varied 2.5-fold at most. The greater variation in the patient with OTC deficiency probably is due to sampling of clusters of normal or abnormal hepatocytes that resulted from inactivation of either the abnormal or normal X chromosome. **Enzyme activity assayed** on small liver biopsy specimens may not be representative of the entire liver in female patients with OTC deficiency. The hyperammonemia in individuals heterozygous for OTC deficiency may be due in part to shunting of blood through multiple 'metabolic portosystemic shunts'. Treatment of a girl who has OTC deficiency with a low-protein diet, a low-protein diet supplemented with oral essential amino acids, and a low-protein diet plus oral ketoacids of essential amino acids was compared in short-term balance studies; on a separate occasion, a low-protein diet was compared to a low-protein diet plus lactulose. The low-protein diet plus oral ketoacid supplementation resulted in the best metabolic **control** of the patient's disease. On the other hand, paradoxical transient hyperammonemia was observed after the intravenous administration of ketoacids to two acutely ill female patients with OTC deficiency.

Inventor Search

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L4 ANSWER 1 OF 1 HCAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:450256 HCAPLUS
DOCUMENT NUMBER: 137:2733
TITLE: Ion-exchange resin / enzyme activity assay
INVENTOR(S): **Karsten, Thomas P.; Currie, Mark G. ; Moore, William M.**
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 7 pp.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002072082	A1	20020613	US 2001-888008	20010622

PRIORITY APPLN. INFO.: US 2000-213354P P 20000622

AB The present invention relates to a rapid high-throughput ion-exchange resin assay for determining enzyme activity. This novel assay uses a radiometric technique which separates the radioactive substrate from the product (or the radioactive product from the substrate) by exploiting the differences in the net charges of the mols. using ion-exchange resin. This assay is useful, for example, for studies of enzyme kinetics, the identification of functional sites in the enzyme, and in the automated screening of compound libraries for pharmaceutical drug development.